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nature

ol 268 No 5615 7 July 1977 55p

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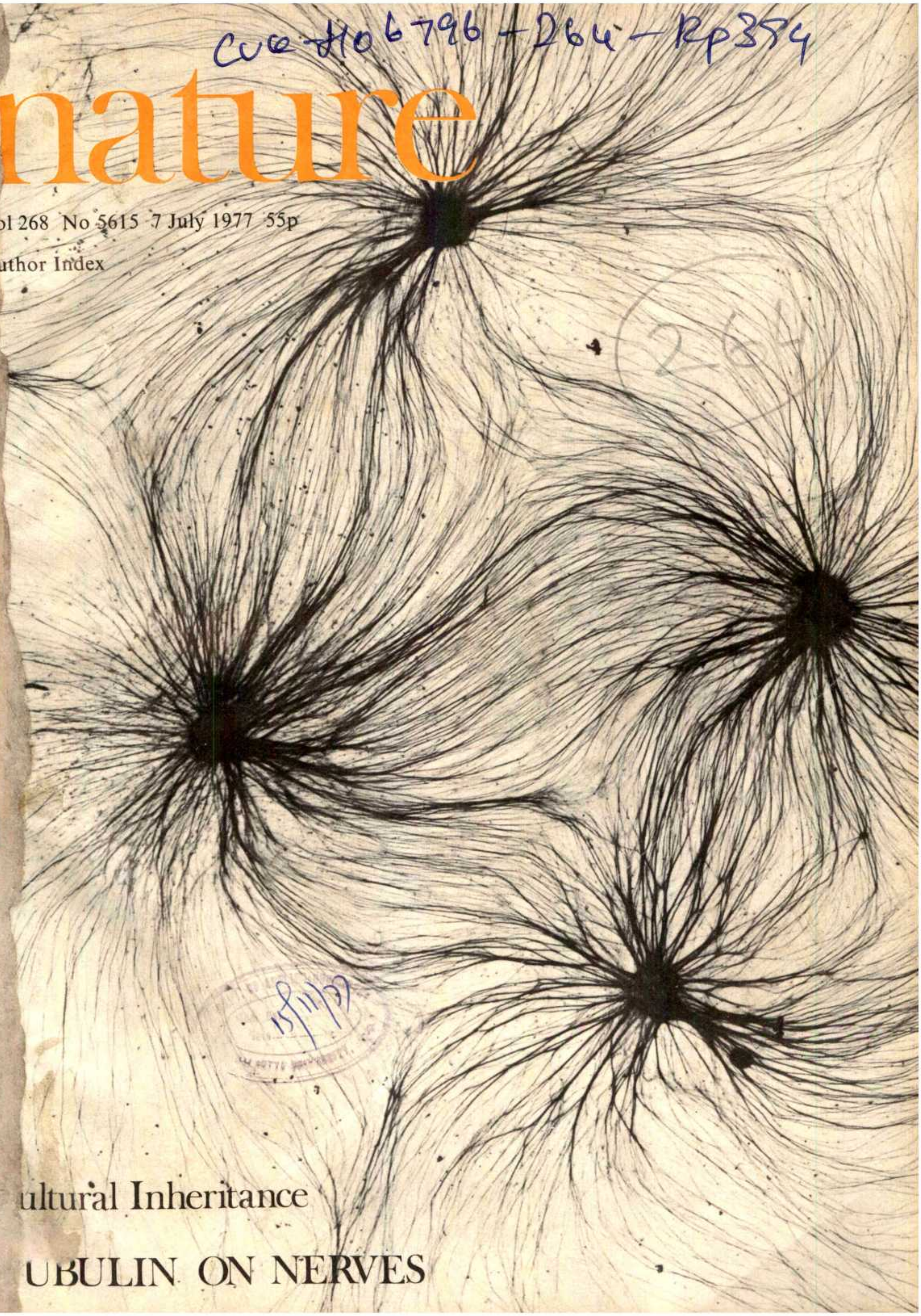

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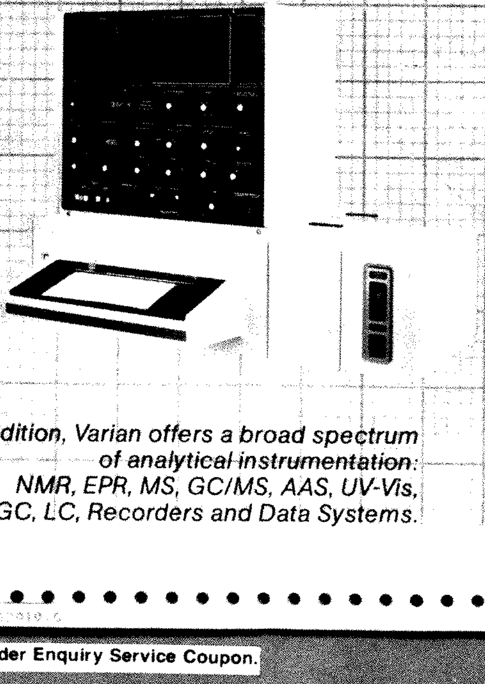

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Cover picture

Superior cervical
ganglia explants
(see page 60)

Vol. 267 No. 5615 **7 July 1977**

nature

Volume 268 7 July 1977

15/11/77

264

Getting at the truth about Dr Marshall 1
The concept of usefulness 2
OTA's stormy ride 4
USA/Asbestos/Czechoslovakia/West Germany/Sweden/In brief/Mellanby on insects 6

NEWS AND VIEWS

Population genetics and cultural inheritance/Seismic shocks in Alaska/ Palaeoecology/ Amyloidosis/ Bitumen sediments/ Oncornavirus RNA/ Carbon lamellar compounds/Laser spectroscopy/Heavy ion transfer reactions 11

REVIEW ARTICLE

Energy production by microbial photosynthesis — *J. R. Benemann, J. C. Weissman, B. L. Koopman and W. J. Oswald* 19

ARTICLES

Lateral mobility of human erythrocyte integral membrane proteins — *V. Fowler and D. Branton* 23
Plate linkage mechanism to account for oroclinal deformation in the Western Cordillera of North America — *W. B. Heptonstall* 27

LETTERS TO NATURE

Uranus rings: an optical search — *B. A. Smith* 32
Observations of Uranus occultation events — *W. B. Hubbard, G. V. Coyne, T. Gehrels, B. A. Smith and B. H. Zellner* 33
Wave optics of the central spot in planetary occultations — *W. B. Hubbard* 34
Video tape recording of a possible ball lightning event — *A. J. Eriksson* 35
Open system crystal fractionation and incompatible element variation in basalts — *R. J. Pankhurst* 36
Palaeoatmospheric argon in Rhynie chert — *P. H. Cadogan* 38
Lead isotope measurements from the oldest recognised Lewisian gneisses of north-west Scotland — *H. J. Chapman and S. Moorbath* 41
Giant late Jurassic sabkhas of Arabian Tethys — *M. R. Leeder and R. Zeidan* 42
Contact angle and film jumps — *J. van Brakel and P. M. Heertjes* 44
Palaeoecological investigations of the site at Hampstead Health, London — *M. Girling and J. Greig* 45
Summer food as the factor limiting roe deer population size — *B. Bobek* 47

Guide to authors

- Review articles should be aimed at a relatively wide readership. Many reviews are invited, but submitted articles may also be accepted; it is advisable to consult us before writing a review article.
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Injury requirement for initiation of regeneration of newt limbs which have whole skin grafts	*R. A. Tassava and R. M. Loyd*	49
Bilateral interaction in olfaction	*W. S. Cain*	50
Bicyclic phosphorus esters increase the cyclic GMP level in rat cerebellum	*H. Mattsson, K. Brandt and E. Heilbronn*	52
A new class of GABA agonist	*P. Krogsgaard-Larsen, G. A. R. Johnston, D. Lodge and D. R. Curtis*	53
Abnormal adenine metabolism of erythrocytes in Duchenne and myotonic muscular dystrophy	*C. C. Solomons, S. P. Ringel, E. I. Nwuke and H. Suga*	55
Amino acid sequence similarities between amyloid P component Clt and CRP	*Y. Levo, B. Frangione and E. C. Franklin*	56
An anterior pituitary factor stimulates thymidine incorporation in isolated thymocytes	*R. K. Saxena and G. P. Talwar*	57
Propranolol increases binding of thyrotropin to thyroid membranes	*N. J. Marshall, S. von Borcke, A. Florin-Christensen and R. P. Ekins*	58
Polypeptides similar to the α and β subunits of tubulin are exposed on the neuronal surface	*M. Estridge*	60
Type I and type II cyclic AMP-dependent protein kinase as opposite effectors of lymphocyte mitogenesis	*C. V. Byus, G. R. Klimpel, D. O. Lucas and D. H. Russell*	63
Chinese hamster cell mutant with defective mitochondrial protein synthesis	*G. Ditta, K. Soderberg and I. E. Scheffler*	64
Interferon production during lymphocytic choriomeningitis virus infection of nude and normal mice	*T. C. Merigan, M. B. A. Oldstone and R. M. Welsh*	67
Immunological tolerance and tumour allografts in the brain	*M. Hasek, J. Chutna, M. Sladecek and Z. Lodin*	68
Macrophages isolated from regressing Moloney sarcomas are more cytotoxic than those recovered from progressing sarcomas	*S. W. Russell and A. T. McIntosh*	69
Aspartic acid racemisation in the human lens during ageing and in cataract formation	*P. M. Masters, J. L. Bada and J. S. Zigler*	71
A role of selenium against methylmercury toxicity	*K. Sumino, R. Yamamoto and S. Kitamura*	73
Intermolecular association and geometrical shape as two factors controlling chemical reactions	*T. Endo, A. Kuwahara, H. Tasai, T. Murata, M. Hashimoto and T. Ishigami*	74
Effects of cyclic AMP pulses on adenylate cyclase and the phosphodiesterase inhibitor of *D. discoideum*	*C. Klein and M. Darmon*	76
Significance of glucose oxidase in lignin degradation	*T. R. Green*	78
Unusual mitosis in the red alga *Porphyridium purpureum*	*R. Bronchart and V. Demoulin*	80
Carotenoid transformations during chloroplast development in *Scenedesmus obliquus* PG1 demonstrated by deuterium labelling	*G. Britton, W. J. S. Lockley, R. Powls, T. W. Goodwin and L. M. Heyes*	81

BOOK REVIEWS

Rabies: The Facts (Colin Kaplan, editor)	*G. M. Baer*	83
Wildfowl of Europe (Myrfyn Owen)	*Peter Conder*	84
Human Biology: An Exhibition of Ourselves (British Museum Natural History)	*Mary Lindley*	84
Genetic Evolution (Chen Kang Chai)	*J. A. Bishop*	85
Handbook of Chemical Lasers (R. W. F. Gross and J. F. Bott, editors)	*Ian Spalding*	85
Behaviour and Misbehaviour: Explanations and Non-Explanations (Nigel Walker)	*Stuart Sutherland*	86
Enzyme Kinetics (D. V. Roberts)	*K. F. Tipton*	86

Newly on the market 87

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See page 124.

Vol. 268 No. 5616 **14 July 1977**

nature

3379

Volume 268 15/xi/77 **14 July 1977**

Million-dollar problem—billion-dollar solution? 89
Twelve months after Séveso 90
Rational containment on recombinant DNA 90
Sulphur deposit account 92
USA/USSR/EEC/JET/Britain/France/China/Bangladesh/In brief/Mellanby on sulphur pollution 94

Correspondence 100

NEWS AND VIEWS

Adenovirus at Cold Spring Harbor/Saturn/Membrane channels/NMR of macromolecules/Neoplastic transformation/Y chromosome DNA 101

REVIEW ARTICLE

Transposable genetic elements as agents of gene instability and chromosomal rearrangements *P. Nevers and H. Saedler* 109

ARTICLES

Protein–DNA and protein–hormone interactions in prealbumin: a model of the thyroid hormone nuclear receptor? *C. C. F. Blake and S. J. Oatley* 115
Separation of membrane currents using a *Paramecium* mutant *D. Oertel, S. J. Schein and C. Kung* 120

LETTERS TO NATURE

Airborne studies of thunderstorm electrification *W. Gaskell, A. J. Illingworth, J. Latham and C. B. Moore* 124
Pattern recognition applied to uranium prospecting *P. L. Briggs and F. Press* 125
An important ground surface sink for atmospheric nitrous oxide *K. A. Brice, A. E. J. Eggleton and S. A. Penkett* 127
Generation of a supported iridium catalyst of extremely high dispersion *J. R. Anderson and R. F. Howe* 129
Hydrogen in the Earth's core *D. J. Stevenson* 130
Geochronology of some blueschists from Ile de Groix, France *J. J. Peucat and J. Cogne* 131
^{40}Ar/^{39}Ar ages of a diabase sill and a basalt in the Central-Pacific Basin *I. Kaneoka and M. Ozima* 132
Mercury emissions from chlorine-production solid waste deposits *S. E. Lindberg and R. R. Turner* 133
Dissolved aluminium concentration in seawater *D. J. Hydes* 136
Optimal foraging in great tits *(Parus major)* *R. J. Cowie* 137
Nature and precursors of juvenile hormone-induced excessive cuticular melanisation in German cockroach *Y. T. Das and A. P. Gupta* 139
Simultaneous recording from several neurones in an invertebrate central nervous system *A. Grinvald, B. M. Salzberg and L. B. Cohen* 140
Antagonism of ethanol narcosis by histidyl-proline diketopiperazine *C. Prasad, T. Matsui and A. Peterkofsky* 142
Eighty thousand β-adrenoreceptors in a single cell *D. Atlas, E. Hanski and A. Levitzki* 144

Guide to authors

- Review articles should be aimed at a relatively wide readership. Many reviews are invited, but submitted articles may also be accepted; it is advisable to consult us before writing a review article.
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How motoneurones control development of muscle tension — *F. Baldissera and P. Campadelli* 146
Acetylcholine-like effects of intracellular calcium application in pancreatic acinar cells — *N. Iwatsuki and O. H. Petersen* 147
Nuclear localisation of an oocyte component required for the stability of injected DNA — *A. H. Wyllie, J. B. Gurdon and J. Price* 150
Rigid control of synthesis of collagen Types I and III by cells in culture — *A. J. Hance and R. G. Crystal* 152
Long term culture of tumour-specific cytotoxic T cells — *S. Gillis and K. A. Smith* 154
Effect of glucose and insulin on collagen secretion by human skin fibroblasts *in vitro* — *D. B. Villee and M. L. Powers* 156
Spontaneous and induced cell-mediated reactivity to syngeneic cells — *D. Senitzer, W. Cafruny, B. Pansky and E. H. Freimer* 158
Inhibition of platelet aggregation by a placental substance with prostacyclin-like activity — *L. Myatt and M. G. Elder* 159
Prostacyclin is the major prostaglandin released from the isolated perfused rabbit and rat heart — *E. A. M. de Deckere, D. H. Nugteren and F. T. Hoor* 160
Transitory expression of Thy-1 antigen in skeletal muscle development — *J. F. Lesley and V. A. Lennon* 163
Relationship between Na : K and Na : Na exchange by the sodium pump of skeletal muscle — *B. G. Kennedy and P. De Weer* 165
Independent mechanisms of cyclic AMP and glucocorticoid action — *U. Gehring and P. Coffino* 167
The shape of the ultraviolet inactivation curve for transforming DNA — *J. K. Setlow* 169
Psoralen and near ultraviolet light: a probe for study of control of protein synthesis — *Y. M. Heimer, E. Ben-Hur and E. Riklis* 170
Genetic recombination through protoplast fusion in *Streptomyces* — *D. A. Hopwood, H. M. Wright, M. J. Bibb and S. N. Cohen* 171
Test of the ballistic hypothesis of movement and the 'point of no return' — *K. Flowers* 174
Lateralised perception of bilateral chimaeric faces by normal subjects — *A. D. Milner and J. J. Dunne* 175

MATTERS ARISING

Dysmyelination in Jimpy mouse due to astroglial hyperlasia? — *C. Meier and A. Bischoff* 177
Reply — *R. P. Skoff* 177
Nature of stromatoporoids — *R. Riding and S. Kershaw* 178
Do antigen-specific helper factors in rabbit lack V-region Ig determinants? — *G. Sundharadas* 178
Reply — *M. J. Taussig, A. P. Finch and A. S. Kelus* 178

BOOK REVIEWS

The Structure of the Universe (Jayant Narlikar) — *Paul Davies* 179
Methods in Receptor Research (M. Blecher) — *H. W. Kosterlitz* 179
Development of Cognitive Processes (V. Hamilton and M. D. Vernon, editors) — *Beate Hermelin* 180
Experimental Embryogenesis in Vascular Plants (V. Raghavan) — *William Newcomb* 180
Magnetic Ions in Metals (R. H. Taylor) — *B. Henderson* 181
Treatment of Industrial Effluents (A. G. Gallely, C. F. Forster and D. A. Stafford, editors) — *P. Coackley* 181
Resource Allocation and Productivity in National and International Agriculture (T. M. Arndt, D. G. Dalrymple and V. W. Ruttan, editors) — *W. S. Wise* 182
Protozoa (A. Westphal) — *R. E. Sinden* 182

Announcements and Person to Person 183

M

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Vol. 268 No. 5617 **21 July 1977**

nature

Volume 268 21 July 1977

15/11/77

Recombinant DNA debate three years on 185
A world fit for whales? 186
Australia: When two academies meet 187
Making paper work 188
USA/Europe/USSR 190

Correspondence and competition 194

NEWS AND VIEWS

Anti-cancer drugs/*lac* repressor/Elephants/Nitrate in water/Insulin recombinant/Expanding Earth 196

REVIEW ARTICLE

Quark confinement — *R. L. Jaffe* 201

ARTICLES

The location of the polio genome protein in viral RNAs and its implication for RNA synthesis — *A. Nomoto, B. Detjen, R. Pozzatti and E. Wimmer* 208
Purification and structural characterisation of human *HLA*-linked B-cell antigens — *T. A. Springer, J. F. Kaufman, C. Terhorst and J. L. Strominger* 213

LETTERS TO NATURE

Water vapour concentrations at the mesopause — *F. Arnold and D. Krankowsky* 218
Tectonic rotation in Western Canada — *A. Hicken and E. Irving* 219
Frequency dependence of elasticity of rock—test of seismic velocity dispersion — *B. J. Brennan and F. D. Stacey* 220
Carboniferous unconformity in southern Africa — *R. P. Stapleton* 222
Silicate and the aqueous corrosion of a high-magnesium alloy — *B. Case, P. M. Bradford and K. Garbett* 223
Thermoluminescence from γ-ray irradiated normal paraffins — *T. Hashimoto, H. Shimada and T. Sakai* 225
Photocell using covalently-bound dyes on semiconductor surfaces — *M. Fujihira, N. Ohishi and T. Osa* 226
Spanish rock art depicting honey gathering during the Mesolithic — *M. Dams and L. Dams* 228
The ornithopod dinosaur *Dryosaurus* and a Laurasia-Gondwanaland connection in the Upper Jurassic — *P. M. Galton* 230
Vesicular-arbuscular mycorrhiza in some aquatic vascular plants — *M. Søndergaard and S. Laegaard* 232
Evolution of long-distance alarm calls in Kloss's gibbon — *R. R. Tenaza and R. L. Tilson* 233
Role of sister chromatid exchanges in chromatid aberration formation — *T. Ikushima* 235
Interferon suppresses the transition of quiescent 3T3 cells to a growing state — *Y. Sokawa, Yoshihiko Watanabe, Yuji Watanabe and Y. Kawade* 236
Inhibition of lymphocyte-induced angiogenesis by isolated chondrocytes — *M. Kaminski, G. Kaminska, M. Jakobisiak and W. Brzezinski* 238
Human smooth muscle autoantibodies reacting with intermediate (100 Å) filaments — *P. Kurki, E. Linder, I. Virtanen and S. Stenman* 240
Myogenic defects in human muscular dystrophy — *E. J. Thompson, R. Yasin, G. V. Beers, K. Nurse and S. Al-Ani* 241

Guide to authors

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A human leukaemic cell expressing hybrid membrane phenotypes — *G. F. Burns, A. A. Nash, C. P. Worman, C. R. Barker, F. G. J. Hayhoe and J. C. Cawley* 243
Epidermal Langerhans cells bear Fc and C3 receptors — *G. Stingl, E. C. Wolff-Schreiner, W. J. Pichler, F. Gschnait, W. Knapp and K. Wolff* 245
Ia antigen expression on human epidermal Langerhans cells — *G. Rowden, M. G. Lewis and A. K. Sullivan* 247
Epidermal Langerhans cells express Ia antigens — *L. Klareskog, U. M. Tjernlund, U. Forsum and P. A. Peterson* 248
Cytotoxicity against tumour-associated antigens not H–2 restricted — *H. T. Holden and R. B. Herberman* 250
Relationship between β-adrenergic receptors and adenylate cyclase in HeLa cells — *R. C. Henneberry, C. C. Smith and J. F. Tallman* 252
Thyroid hormone controls glucocorticoid action in cultured GH_1 cells — *H. H. Samuels, Z. D. Horwitz, F. Stanley, J. Casanova and L. E. Shapiro* 254
Muscarinic antagonists block cone to horizontal cell transmission in turtle retina — *H. M. Gerschenfeld and M. Piccolino* 257
Lateral interactions in the outer plexiform layer of turtle retinas after atropine block of horizontal cells — *M. Piccolino and H. M. Gerschenfeld* 259
Membrane control of cardiac contractility — *G. B. McClellan and S. Winegrad* 261
Postsynaptic effects of a multiaction giant interneurone on identified snail neurones — *W. Winlow and P. R. Benjamin* 263
Spontaneous release of transmitter from 'repressed' nerve terminals in axolotl muscle — *A. J. Harris, L. Ziskind and D. Wigston* 265
Are transmitter release statistics meaningful? — *S. B. Barton and I. S. Cohen* 267
Replication of EBV in epithelial cells during infectious mononucleosis — *S. M. Lemon, L. M. Hutt, J. E. Shaw, J.-L. H. Li and J. S. Pagano* 268
5′-Terminal nucleotide sequences of polio virus polyribosomal RNA and virion RNA are identical — *R. F. Pettersson, J. B. Flanegan, J. K. Rose and D. Baltimore* 270

BOOK REVIEWS

Darwin and His Flowers: The Key to Natural Selection (Mea Allan) — *R. H. Richens* 273
The Correspondence of Isaac Newton (Rupert A. Hall and Laura Tilling, editors) — *J. W. Herivel* 273
Light and Life (Lars Olaf Björn) — *P. D. Moore* 274
Digital Image Restoration (H. C. Andrews and B. R. Hunt); Digital Picture Analysis (A. Rosenfeld, editor); Image Science Mathematics (C. O. Wilde and E. Barrett, editors) — *P. W. Hawkes* 275
Comparative Immunology (J. L. Marchalonis, editor) — *J. D. Horton* 275
Liquids and Solutions: Structures and Dynamics (P. Kruus) — *H. N. V. Temperley* 276
Intelligence: Nature, Determinants and Consequences (E. B. Brody) — *A. D. B. Clarke* 276

Obituaries

P. Edman — *H D Niall* 279
C. J. Smithells — *G. L. Bailey* 280
R. N. Singh — *P. K. Singh and G E. Fogg* 280
E. Lilly — *F. Hartley* 280

Announcements and Person to Person 281

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Cover picture

Professor Ambraseys writes on some of the effects of the recent Romanian earthquake on page 324

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Vol. 268 No. 5618 **28 July 1977**

nature

Volume 268 **28 July 1977**

Peking exacts too high a price 283
Cancer prevention: a question of priorities 284
Soviet genetics: new controversy 285
ILL at ease 287
USA/Britain/Backgrounder/In brief/ Jukes on COSPAR 289

Correspondence 294

NEWS AND VIEWS

Muon lifetime/Carbon dioxide and forests/New quarks/Levich conference/Ocean magnetism/Magnesium 295

ARTICLES

Measurements of relativistic time dilation for positive and negative muons in a circular orbit	*J. Bailey, K. Borer, F. Combley, H. Drumm, F. Krienen, F. Lange, E. Picasso, W. von Ruden, F. J. M. Farley, J. H. Field, W. Flegel and P. M. Hattersley*	301
Rifting and subsidence on passive continental margins in the North East Atlantic	*L. Montadert, D. G. Roberts, G. A. Auffret, W. Bock, P. A. DuPeuble, E. A. Hailwood, W. Harrison, H. Kagami, D. N. Lumsden, C. Muller, D. Schnitker, R. W. Thompson, T. L. Thompson and P. P. Timofeev*	305
Coupling of hormone receptors to adenylate cyclase of different cells by cell fusion	*M. Schramm, J. Orly, S. Eimerl and M. Korner*	310
Self-assembly and catalytic activity of the pyruvate dehydrogenase multienzyme complex of *Escherichia coli*	*D. L. Bates, M. J. Danson, G. Hale, E. A. Hooper and R. N. Perham*	313

LETTERS TO NATURE

NP0532 and a hole in the Crab Nebula	*R. Isaacman,*	317
The optical variability of 3C345	*C. Barbieri, G. Romano, S. di Serego and M. Zambon*	318
Small-amplitude, short-period waves in the E and F regions of the ionosphere	*J. C. Devlin, P. L. Dyson and P. R. Hammer*	319
Radioactive ^{38}S in an atmospheric SO_2 and SO_2 oxidation rate measurement	*W. Roedel, W. Junkerman and L. Leidner*	320
Stresses at spherulite boundaries	*P. D. Calvert*	321
Transport of metal complex chlorides through plastic film membrane	*H. D. Gesser, G. A. Horsfall, K. M. Gough and B. Krawchuk*	323
Long-period effects in the Romanian earthquake of March 1977	*N. N. Ambraseys*	324
Confirmation of central volcanoes off the Icelandic coast	*L. Kristjansson, K. Thors, and H. R. Karlsson*	325
How do children learn the meaning of verbs?	*T. Wykes and P. N. Johnson-Laird*	326
Adoption study supporting genetic transmission in manic–depressive illness	*J. Mendlewicz and J. D. Rainer*	327
Number of trophic levels in ecological communities	*S. L. Pimm and J. H. Lawton*	329
Large benthic microbial communities in sulphide biota under Peru–Chile Subsurface Countercurrent	*V. A. Gallardo*	331
Drinking behaviour induced by intracranial injections of eledoisin and substance P in the pigeon	*M. D. Evered, J. T. Fitzsimons and G. de Caro*	332
Cells selective to binocular disparity in the cortex of newborn lambs	*V. S. Ramachandran, P. G. H. Clarke and D. Whitteridge*	333

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Nature's publishing policy is outlined in **258,** 1 (1975) and **264,** v, 11 Nov. (1976).

Early hyperthyroidism alters the distribution of mossy fibres in the rat hippocampus — *J. M. Lauder and E. Mugnaini* 335
Generality of oestrogen stimulation of peroxidase activity in growth-responsive tissues — *C. R. Lyttle and E. R. DeSombre* 337
Comparative drug sensitivities of culture forms of *Trypanosoma cruzi* and *Trypanosoma dionisii* — *M. Gaborak, J. L. Darling and W. E. Gutteridge* 339
Eosinophils and not lymphoid K cells kill *Trypanosoma cruzi* epimastigotes — *C. J. Sanderson, A. F. Lopez and M. M. Bunn Moreno* 340
Type C virus expression and host response in diet-cured NZB/W mice — *M. B. Gardner, J. N. Ihle, R. J. Pillarisetty, N. Talal, E. L. Dubois and J. A. Levy* 341
Epizootiology, transmission and approach to prevention of fatal simian haemorrhagic fever in rhesus monkeys — *W. T. London* 344
Y-forms as possible intermediates in the replication of measles virus nucleocapsids — *H. V. Thorne and E. Dermott* 345
Presence of 1,25-dihydroxyvitamin D_3-glycoside in the calcinogenic plant *Cestrum diurnum* — *M. R. Hughes, T. A. McCain, S. Y. Chang, M. R. Haussler, M. Villareale and R. H. Wasserman* 347
Synthesis, storage and release of acetylcholine by a noradrenergic pheochromocytoma cell line — *L. A. Greene and G. Rein* 349
Substance P and opiate receptors — *J. Davies and A. Dray* 351
Evidence for a cyclic nucleotide-mediated calcium flux in motor nerve terminals — *L. R. Skirboll, L. Baizer and K. L. Dretchen* 352
A modifier of $(Na^+ + K^+)$ ATPase in commercial ATP — *L. A. Beaugé and I. M. Glynn* 355
The molecular mechanisms of anaesthesia — *D. A. Haydon, B. M. Hendry, S. R. Levinson and J. Requena* 356
Membrane asymmetry and blood coagulation — *R. F. A. Zwaal, P. Comfurius and L. L. M. van Deenen* 358
Ion transport and rotation of bacterial flagella — *P. Läuger* 360
Analysis of positional information applied to cirral patterns of the ciliate *Euplotes* — *M. A. Gates* 362
Ion-adsorbent substomatal structures in *Tradescantia pallidus* — *R. A. Stevens and E. S. Martin* 364
Heterogeneous population of mitochondrial DNA molecules in higher plants — *F. Quétier and F. Vedel* 365
Inhibition by L-ascorbate of bacterial mutagenesis induced by two *N*-nitroso compounds — *J. B. Guttenplan* 368
Intracellular cationic counterion composition of an acid mucopolysaccharide — *S. Hunt and K. Oates* 370
Pressure effects on proton tunnelling — *N. S. Isaacs, K. Javaid and E. Rannala* 372

MATTERS ARISING

Generalisation of self-thinning of plant populations — *J. White* 373
Reply — *M. Westoby* 373
Atlantic palaeotemperatures during the Cainozoic — *D. G. Jenkins* 373
Reply — *R. Cifelli* 374
U-shaped gene frequency distributions — *G. Bell* 374
Reply — *A. Ondricek, E. A. Gilpin, M. E. Soule and M. E. Gilpin* 374

BOOK REVIEWS

Evolution and the Diversity of Life: Selected Essays (Ernst Mayr) — *A. J. Cain* 375
Transport in Plants (C. R. Stocking and U. Heber, editors) — *J. L. Hall* 376
Point Defects in Crystals (R. K. Watts) — *John Walker* 376
Volcanoes of the Earth (Fred M. Bullard) — *Peter Francis* 377
Evolution of the Crystalline Rocks (D. K. Bailey and R. Macdonald, editors) — *J. Nolan* 377
Atomic Processes and Applications (P. G. Burke and B. L. Moiseiwitsch, editors) — *J. C. Gunn* 378
Mission to Earth: Landsat Views the World (N. M. Short, P. D. Lowman, Jr, S. C. Freden and W. A. Finch, Jr) — *P. Schagen* 378

Obituary – W. Grey Walter; Frank Green; M. F. Lucas Keene 383

M

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Cover picture

On page 421, S. Soter and A. Harris discuss a possible cause of these curious features on Phobos.

Vol. 268 No. 5619 **4 August 1977**

nature

Volume 268 4 August 1977

Test ban: new hopes, new hurdles 385
What kind of food policy? 386
Britain/JET/Europe/USA/Canada/In brief/Mellanby on population 388
Correspondence 396

NEWS AND VIEWS

Gravity experiments/Translating tumour viruses/Amorphous semiconductors/Muscle contraction/Cosmic ray age/Crop protection/Tree-ring dating/Tongue sole taxonomy 397

REVIEW ARTICLE

Radio sources with superluminal velocities *M. H. Cohen, K. I. Kellermann, D. B. Shaffer, R. P. Linfield, A. T. Moffet, J. D. Romney, G. A. Seielstad, I. I. K. Pauliny-Toth, E. Preuss, A. Witzel, R. T. Schilizzi and B. J. Geldzahler* 405

ARTICLES

Percolation theory of residual phases in porous media *R. G. Larson, L. E. Scriven and H. T. Davis* 409
An 8,000-yr palaeoclimatic record of the 'Double-Hale' 45-yr solar cycle *R. W. Fairbridge and C. Hillaire-Marcel* 413
The size of Rous sarcoma virus mRNAs active in cell-free translation *T. Pawson, R. Harvey and A. E. Smith* 416

LETTERS TO NATURE

Cyg X-3 γ rays from a magnetic accretion disk *P. Mészáros, F. Meyer and J. E. Pringle* 420
Are striations on Phobos evidence for tidal stress? *S. Soter and A. Harris* 421
Geomagnetic storms and electric fields in the equatorial ionosphere *R. G. Rastogi* 422
Bimodal distribution of creep event amplitudes on the San Andreas fault, California *R. O. Burford* 424
Loss of boron from shells during weathering and possible implications for the determination of palaeosalinity *P. J. Cook* 426
Pre-melting near crystal dislocations *F. J. Bartis* 427
Major genes for copper tolerance in *Mimulus guttatus* *M. R. Macnair* 428
Association of alleles at esterase loci in wild barley *Hordeum spontaneum* L. *A. Brown, E. Nevo and D. Zohary* 430
Prediction of seed longevity at sub-zero temperatures and genetic resources conservation *E. H. Roberts and R. H. Ellis* 431
Night flight towards a sex pheromone source by male *Spodoptera littoralis* (Boisd.) (Lepidoptera, Noctuidae) *J. Murlis and B. W. Bettany* 433
Effect of regeneration on compartment specificity of the bithorax mutant of *Drosophila melanogaster* *S. Y. Tiong, J. R. Girton, P. H. Hayes and M. A. Russell* 435
Application of magnetic microspheres in labelling and separation of cells *R. S. Molday, S. P. S. Yen and A. Rembaum* 437
Formation and resealing of pores of controlled sizes in human erythrocyte membrane *K. Kinosita, Jr and T. Y. Tsong* 438
Activation of mouse spleen cells by a single short pulse of mitogen *S. M. Milner* 441

Guide to authors

- Review articles should be aimed at a relatively wide readership. Many reviews are invited, but submitted articles may also be accepted; it is advisable to consult us before writing a review article.
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Development of mast cells from grafted bone marrow cells in irradiated mice — *Y. Kitamura, M. Shimada, K. Hatanaka and Y. Miyano* 442
Evidence for Fc receptors on rabbit yolk sac endoderm — *A. E. Wild and P. Dawson* 443
Localisation of gonadotropin-releasing hormone in the circumventricular organs of human brain — *E. Okon and Y. Koch* 445
Chemical carcinogens stimulate canine kidney (MDCK) cells to produce prostaglandins — *L. Levine* 447
Infectious primate type-C virus shed by healthy gibbons — *T. G. Kawakami, L. Sun and T. S. McDowell* 448
Spatial summation and light adaptation in the goldfish visual system — *D. P. M. Northmore* 450
Stability of Met-enkephalin content in brain structures of morphine-dependent or foot shock-stressed rats — *W. Fratta, H.-Y. T. Yang, J. Hong and E. Costa* 452
Comparison of specificity of agonist and antagonist radioligand binding to β-adrenergic receptors — *R. J. Lefkowitz and M. Hamp* 453
Development of β-adrenergic receptor subsensitivity by antidepressants — *S. P. Banerjee, L. S. Kung, S. J. Riggi and S. K. Chanda* 455
Formation of synapses between parasympathetic neurones deprived of preganglionic innervation — *P. B. Sargent and M. J. Dennis* 456
Synaptic and septate neuromuscular junctions in embryonic lobster muscle — *F. Lang* 458
Is recombination confined to structural genes on the eukaryotic genome? — *P. Thuriaux* 460
n-Butyrate causes histone modification in HeLa and Friend erythroleukaemia cells — *M. G. Riggs, R. G. Whittaker, J. R. Neumann and V. M. Ingram* 462
Yeast virus-like particles possess a capsid-associated single-stranded RNA polymerase — *A. J. Herring and E. A. Bevan* 464
Enzymology in supercooled water — *P. Douzou, P. Debey and F. Franks* 466

Corrigenda 466

BOOK REVIEWS

The Moral Status of Animals (Stephen R. L. Clarke) — *Eric Ashby* 467
Cell Surface Reviews, Volume 1 (G. Poste and G. L. Nicholson, editors) — *Joan Feldman* 467
The Solar Planets (V. A. Firsoff) — *David W. Hughes* 468
Reminiscence, Motivation and Personality (H. J. Eysenck and C. D. Frith) — *Leon J. Kamin* 468
An Introduction to Regge Theory and High Energy Physics (P. D. B. Collins) — *I. G. Halliday* 469
The Chemistry of Mercury (C. A. McAuliffe, editor) — *A. G. Massey* 469
The Chemical Physics of Surfaces (S. Roy Morrison) — *D. Tabor* 470
Our Changing Planet (John Gribbin) — *D. H. Tarling* 470

Obituary

A. L. Thomson — *Harold Himsworth* 471
M. Thomas — *S. L. Ranson* 472
A. V. Nikolaev — *Vera Rich* 472
C. W. F. McClare — *W. R. Lieb and I. Gonda* 472

Newly on the Market 473

M

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Cover picture

The resemblance between geometric motifs in weaving and pottery and the folding patterns of the β-sheets of globular proteins. β-sheet topology is surveyed on pages 495-500.

Vol. 268 No. 5620 11 August 1977

nature

Volume 268 11 August 1977

A suitable case for education 475
The world balance: judge with care 476
Man-made process 478
USA/USSR/EEC/Argentina 480

Correspondence 482

NEWS AND VIEWS

Enzymoimmunoassay/X-ray stars/Enzyme mechanisms/Cometary missions/ Soil structure project/Environmental mutagenesis/Genetic recombination 483

REVIEW ARTICLE

Interstellar molecules *B. Zuckerman* 491

ARTICLES

β-Sheet topology and the relatedness of proteins *J. S. Richardson* 495
Nerve growth factor-induced increase in electrical excitability and acetylcholine sensitivity of a rat pheochromocytoma cell line *M. A. Dichter, A. S. Tischler and L. A. Greene* 501
Large number hypothesis and continuous creation cosmologies *I. W. Roxburgh* 504

LETTERS TO NATURE

A line feature at 64 keV in the X-ray spectrum of Her X-1 *M. J. Coe, A. R. Engel, J. J. Quenby and C. S. Dyer* 508
Relation between metallicity and multiplicity for solar type stars *D. C. Barry* 509
Cosmic rays and ancient catastrophes *J. Wdowczyk and A. W. Wolfendale* 510
Tunguska's comet and non-thermal ^{14}C production in the atmosphere *J. C. Brown and D. W. Hughes* 512
Antarctic topography from balloons *N. Levanon, P. R. Julian and V. E. Suomi* 514
Evaporites from the lower Proterozoic of the East Arm, Great Slave Lake *J. P. N. Badham and C. W. Stanworth* 516
Polycyclic aromatic hydrocarbons in long-range transported aerosols *G. Lunde and A. Bjorseth* 518
The microbe thermistor *B. Mattiasson, P.-O. Larsson and K. Mosbach* 519
Interspecific competition between great and blue tit *A. A. Dhondt* 521
Cercal ablation modifies tethered flight behaviour of cockroach *P. J. Fraser* 523
Transfer of mitochondria by protoplast fusion in *Saccharomyces cerevisiae* *L. Ferenczy and A. Maráz* 524
Circular linkage map of *Rhizobium meliloti* chromosome *A. Kondorosi, G. B. Kiss, T. Forrai, E. Vincze and Z. Banfalvi* 525
Very close linkage between HLA-B and Bf inferred from allelic association *A. Arnason, B. Larsen, W. H. Marshall, J. H. Edwards, P. Mackintosh, B. Olaisen and P. Teisberg* 527
Factors from 3T3 cells stimulate proliferation of cultured vascular endothelial cells *C. R. Birdwell, D. Gospodarowicz and G. L. Nicolson* 528
A T cell-replacing factor specific for histocompatibility antigens in mice *B. Kindred and R. B. Corley* 531
Magnitude of memory to the major histocompatibility complex *B. M. Hall, B. J. Roser and S. E. Dorsch* 532
Sex steroid hormone regulation of IgA and IgG in rat uterine secretions *C. R. Wira and C. P. Sandoe* 534

Guide to authors

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Chemical structure of attachment sites for viruses on human erythrocytes — *B. J. Enegren and A. T. H. Burness* 536
Synthesis of low molecular weight inhibitor of protein synthesis with enzyme from interferon-treated cells — *A. G. Hovanessian, R. E. Brown and I. M. Kerr* 537
Nature of inhibitor of cell-free protein synthesis formed in response to interferon and double-stranded RNA — *I. M. Kerr, R. E. Brown and A. G. Hovanessian* 540
Effects of sodium butyrate on synthesis on human chorionic gonadotrophin in trophoblastic and non-trophoblastic tumours — *J. Y. Chou, J. C. Robinson and S-S. Wang* 543
Potent prolactin and growth hormone releasing activity of more analogues of Met-enkephalin — *L. Cusan, A. Dupont, G. S. Kledzik, F. Labrie, D. H. Coy and A. V. Schally* 544
A synthetic enkephalin analogue with prolonged parenteral and oral analgesic activity — *D. Roemer, H. H. Buescher, R. C. Hill, J. Pless, W. Bauer, F. Cardinaux, A. Closse, D. Hauser and R. Huguenin* 547
Opiate analgesics inhibit substance P release from rat trigeminal nucleus — *T. M. Jessell and L. L. Iversen* 549
Alterations in dopamine receptor sensitivity by chronic ethanol treatment — *P. L. Hoffman and B. Tabakoff* 551
Desensitisation does not selectively alter sodium channels — *D. H. Lambert, P. M. Spannbauer and R. L. Parsons* 553
Significance of extensive 'leaky' cell junctions in the avian salt gland — *R. A. Ellis, C. C. Goertemiller, Jr and D. L. Stetson* 555
Composition of sarcoplasmic reticulum *in situ* by electron probe X-ray microanalysis — *A. V. Somlyo, H. Shuman and A. P. Somlyo* 556
Myoblast myosin phosphorylation is a prerequisite for actin activation — *S. P. Scordilis and R. S. Adelstein* 558
A synthetic polypeptide with antifreeze activity — *V. S. Ananthanarayanan and C. L. Hew* 560
Effect of light on artificial lipid membranes modified by photoreceptor membrane fragments — *E. E. Fesenko and A. L. Lyubarskiy* 562
Effect of aminoacylation on the conformation of yeast phenylalanine tRNA — *R. Potts, M. J. Fournier and N. C. Ford* 563

MATTERS ARISING

The Star of Bethlehem — *O. Edwards, S. Campbell C. M. Botley, N. R. Flores T. J. Palmer and E. V. Hulse* 565
Reply — *D. W. Hughes* 566
Sodium and chloride in rainwater — J. T. Hutton 568
Reply — M. P. Paterson 568
Habitat values and endemicity in the vanishing rain forests of Sri Lanka — *F. R. Senanayake* 568

BOOK REVIEWS

Space and Time in the Modern Universe (P. C. W. Davies) — *D. J. Raine* 569
Astronomy: Principles and Practice/Astronomy: Structure of the Universe (A. E. Roy and D. Clarke) — *Bernard Pagel* 570
Cell Surface Reviews, Volume 2 (G. Poste and G. L. Nicolson, editors) — *P. C. Doherty* 570
Mammalian Cell Membranes, Volumes 4 and 5 (G. A. Jamieson and D. M. Robinson, editors) — *R. C. Hughes* 571
Handbook of Unusual Phenomena (W. R. Corliss) — *John Gribbin* 571
Advances in Computer Chess (M. R. B. Clarke, editor) — *N. J. Holloway* 571
Semimetals and Narrow-Bandgap Semiconductors (D. R. Lovett, editor) — *M. E. Ertl* 572
Spectrochemical Analysis of Pure Substances (Kh. I. Zil'berstein, editor) — *T. S. West* 572
The Year in Endocrinology, 1975-1976 (S. H. Ingbar, editor) — *T. Chard* 572
Announcements 575

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A sunflower illustrated in Besler's *Hortus eystettensis,* volume II, published in 1613; reproduced by permission of the Linnean Society of London. The origin and evolution of cultivated sunflowers and other crop plants in the New World is the subject of a review by Barbara Pickersgill (see p. 591).

Vol. 268 No. 5621 18 August 1977

nature

Volume 268 18 August 1977

15/8/77

A message for them . . . or for us? 577
Heading for Honolulu 578
Taiwan/Sweden/Netherlands/In brief/ Jukes on Berkeley women 580

NEWS AND VIEWS

Optimal foraging/Cosmic ray composition/Ribosomal switches/ New sulphide mineral/Pion production/Blue vision/ Cave ecology/Lampbrush chromosomes 583

REVIEW ARTICLE

Taxonomy and the origin and evolution of cultivated plants in the New World *B. Pickersgill* 591

ARTICLES

Oceanic residence times of trace metals *J. M. Bewers and P. A. Yeats* 595
DNA arrangement in isometric phage heads *W. C. Earnshaw and S. C. Harrison* 598
Initiation of chick cell division by trypsin action at the cell surface *D. H. Carney and D. D. Cunningham* 602

LETTERS TO NATURE

The binary pulsar observed at four radio frequencies *A. G. Lyne and R. T. Ritchings* 606
X-ray bursts from dense clouds *A. C. Fabian* 607
Continuous increase of Hubble modulus behind clusters of galaxies *L. Nottale and J. P. Vigier* 608
Polysaccharides and infrared spectra of galactic sources *F. Hoyle and N. C. Wickramasinghe* 610
Mechanism for formaldehyde polymer formation in interstellar space *V. I. Goldanskii* 612
Dust clouds and frictional generation of glow discharges on Mars *A. A. Mills* 614
Daytime ionosphere scintillation associated with geomagnetic storms *M. R. Deshpande, H. O. Vats, G. Sethia and B. S. Murthy* 614
Volcanic dust veils and North Atlantic climatic change *P. M. Kelly* 616
Oxygen isotope stratigraphy of Late Pleistocene coral terraces in Barbados *N. J. Shackleton and R. K. Matthews* 618
Biological markers in coal and coal liquefaction products *C. M. White, J. L. Shultz and A. G. Sharkey* 620
Stratigraphy of buried soil at Teindland Forest, Scotland *J. C. C. Romans* 622
An Ediacara-type fauna from South Wales *J. C. W. Cope* 624
Tachigalia versicolor is a suicidal neotropical tree *R. B. Foster* 624
Cross-fertilisation in fucoid seaweeds *G. P. Bolwell, J. A. Callow, M. E. Callow and L. V. Evans* 626
Hedgehogs use toad venom in their own defence *E. D. Brodie* 627
Diets of early Miocene African hominoids *R. F. Kay* 628
Further study of an auditory illusion *I. P. Christensen and A. H. Gregory* 630
Racial differences in the frequency of Q and C chromosomal heteromorphisms *H. A. Lubs, W. J. Kimberling, F. Hecht, S. R. Patil, J. Brown, P. Gerald and R. L. Summitt* 631
Androgenetic origin of hydatidiform mole *T. Kajii and K. Ohama* 633
Genetic model for observed distributions of proportions of haemoglobin in sickle-cell trait *G. Brittenham* 635

Guide to authors

- Review articles should be aimed at a relatively wide readership. Many reviews are invited, but submitted articles may also be accepted; it is advisable to consult us before writing a review article.
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Absolute visual threshold and scotopic spectral sensitivity in the tawny owl *Strix aluco* — *G. R. Martin* 636
Maternal effects determine effective lethal phase of *carnation-light* synthetic lethal in *Drosophila melanogaster* — *H. Nickla* 638
Age- and region-dependent concanavalin A reactivity of chick wing-bud mesoderm cells — *D. F. Paulsen and R. A. Finch* 639
Monogamous T helper cell — *J. M. Phillips and H. Waldmann* 641
Dissociation of effector functions in populations of activated macrophages — *E. J. Wing, I. D. Gardner, F. W. Ryning and J. S. Remington* 642
Antiviral T cell-mediated immunity in Marek's disease — *L. J. N. Ross* 644
Heterospecific cytotoxic cell activity induced during the first three days of acute lymphocytic choriomeningitis virus infection in mice — *R. M. Welsh, Jr and R. M. Zinkernagel* 646
Protein covalently linked to foot-and-mouth disease virus RNA — *D. V. Sangar, D. J. Rowlands, T. J. R. Harris and F. Brown* 648
Participation of DNA-dependent RNA polymerase II in replication of influenza viruses — *L. L. R. Spooner and R. D. Barry* 650
Dopamine selectively increases ^{3}H-GABA release from slices of rat substantia nigra *in vitro* — *J.-C. Reubi, L. L. Iversen and T. M. Jessell* 652
Müller cell localisation of glutamine synthetase in rat retina — *R. E. Riepe and M. D. Norenburg* 654
Electrical stimulation of denervated muscles reduces incorporation of methionine into the ACh receptor — *Z. W. Hall and C. G. Reiness* 655
High conductance in an epithelial membrane not due to extracellular shunting — *J. Augustus, J. Bijman, C. H. van Os and J. F. G. Slegers* 657
Fluorescent probe detects redistribution of cell calcium during stimulus–secretion coupling — *D. E. Chandler and J. A. Williams* 659
Evidence for an α-ecdysone cytochrome P-450 mixed function oxidase in insect fat body mitochondria — *W. E. Bollenbacher, S. L. Smith, J. J. Wielgus and L. I. Gilbert* 660
Potential-dependent transition temperature of ionic channels induced by glutamate in locust muscle — *C. R. Anderson, S. G. Cull-Candy and R. Miledi* 663
Are cell affinity relationships for insect epidermal tissues evidence for combinatorial control? — *J. B. Nardi* 665
Control of induction of tubulin synthesis in *Chlamydomonas reinhardi* — *D. P. Weeks, P. S. Collis and M. A. Gealt* 667

MATTERS ARISING

Geomagnetism and climate — *A. D. Hecht* 669
Palaeomagnetic stratigraphy of the Koobi Fora Formation, east of Lake Turkana (Lake Rudolf), Kenya — *J. M. Harris* 669
Reply — *J. Hillhouse* 670
Maya dates AD 352–1296 — *D. J. Schove* 670

BOOK REVIEWS

The Nature of Science: The History of Science in Western Culture Since 1600 (David Knight) — *J. R. Ravetz* 671
Molecular Aspects of Gene Expression in Plants (J. A. Bryant, editor) — *Daphne J. Osborne* 671
Cognitive Development: Its Cultural and Social Foundations (A. R. Luria) — *Jerome Bruner* 672
Intermolecular Interactions and Biomolecular Organisation (A. J. Hopfinger) — *Henryk Eisenberg* 673
Science and Government Report International Almanac, 1977 (Daniel S. Greenberg, editor) — *Stuart S. Blume* 673
Gene Activity in Early Development (E. H. Davidson) — *J. B. Gurdon* 674
Ecological Sanity (George Claus and Karen Bolander) — *Kenneth Mellanby* 674

Obituary 675

M

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Cover picture

Diffraction patterns obtained during microscope calibration. See 'Molecular images by electron-wave holography' on page 707.

Vol. 268 No. 5622 **25 August 1977**

nature

Volume 268 25 August 1977

17/11/77

Believing in OSTP's merits 681
Science in the Himalayas 682
Earthquakes/Denmark/Comecon/In brief/Mellanby on energy and agriculture 684

NEWS AND VIEWS
Mantle convection/Bedbugs and hepatitis/Antibody multispecificity/Nucleus–nucleus collisions/Meteorite research/Enzyme regulation in plant cells 687

REVIEW ARTICLE
Why the genome does not congeal — *J. Maynard Smith* 693

ARTICLES
Antarctic circumpolar current: space and time fluctuations in the Drake Passage — *D. J. Baker, Jr, W. D. Nowlin, Jr, R. D. Pillsbury and H. L. Bryden* 696
New Miocene locality in Turkey with evidence on the origin of *Ramapithecus* and *Sivapithecus* — *P. Andrews and H. Tobien* 699
A genetic model for sympatric speciation through habitat diversification and seasonal isolation — *C. A. Tauber and M. J. Tauber* 702

LETTERS TO NATURE
Variable mid-latitude X-ray source 3U0042+32 — *S. Rappaport, G. W. Clark, R. Dower, R. Doxsey, G. Jernigan and F. Li* 705
Supernovae and molecular clouds — *J. C. Wheeler and F. N. Bash* 706
Molecular images by electron-wave holography — *L. S. Bartell and R. D. Johnson* 707
Formation of silver mirror by photolysis of thin films of high molecular weight silver compounds — *Y. Konishi, H. Saijo, H. Hada and M. Tamura* 709
Particulate transport through the mesosphere and stratosphere — *L. C. Hale* 710
Novel method for direct analysis of hydrocarbons in crime investigation and air pollution studies — *J. D. Twibell and J. M. Home* 711
The Asian source of Arctic haze bands — *K. A. Rahn, R. D. Borys and G. E. Shaw* 713
Sulphur dioxide discharge from Mount Etna — *R. Haulet, P. Zettwoog and J. C. Sabroux* 715
Superheavy element fission hypothesis in carbonaceous chondrites — *G. J. Flynn and M. Loubet* 717
An early Cambrian chert biota and its implications — *M. D. Brasier* 719
Continental margin fault pattern mapped south-west of Ireland — *R. V. Dingle and R. A. Scrutton* 720
Holocene variations in the level of Lake Bosumtwi, Ghana — *M. R. Talbot and G. Delibrias* 722
Final results of study of infants at risk of sudden death — *R. G. Carpenter and J. L. Emery* 724
Hypolimnas bolina (L.), a mimic of danaid butterflies, and its model *Euploea core* (Cram.) store cardioactive substances — *N. A. Marsh, C. A. Clarke, M. Rothschild and D. N. Kellett* 726
Decay in juvenile hormone biosynthesis by insect corpus allatum after nerve transection — *S. S. Tobe, C. S. Chapman and G. E. Pratt* 728
Sex hormone of *Dictyostelium discoideum* is volatile — *K. E. Lewis and D. H. O'Day* 730
Modification of circadian flight activity in the mosquito *Anopheles gambiae* after insemination — *M. D. R. Jones and S. J. Gubbins* 731
2,4-D plasmids and persistence — *J. M. Pemberton and P. R. Fisher* 732

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Articles should be accompanied by an abstract of not more than fifty words. Letters should begin with a paragraph giving the background and main conclusions in terms intelligible to as wide a readership as possible.

Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the Système International. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible; exp (a) is preferred to e^a if 'a' is more than one character.

References are indicated by superscripts in the text. See any contemporary *Nature* for style, but note:

(i) only one reference number need be used if the reference is to several papers by identical authors,

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An antiviral substance extracted from *Streptococcus faecalis*	*S. Hotta, M. Kojima, M. Fujisaki, S. Uchida, H. Kuroda and C. Hamada*	733
An immunological basis for inhibition of transformation of human lymphocytes by EB virus	*D. J. Moss, W. Scott and J. H. Pope*	735
Intermittent exposure to fluorescent light extends lifespan of human diploid fibroblasts in culture	*R. Parshad and K. K. Sanford*	736
Contact-mediated communication of ouabain resistance in mammalian cells in culture	*C. M. Corsaro and B. R. Migeon*	737
Synergism between anti-microtubule agents and growth stimulants in enhancement of cell cycle traverse	*M-H. Teng, J. C. Bartholomew and M. J. Bissell*	739
5-Hydroxytryptamine and punishment	*N. C. Tye, B. J. Everitt and S. D. Iversen*	741
Intranigral kainic acid is evidence that nigral non-dopaminergic neurones control posture	*G. Di Chiara, M. Olianas, M. Del Fiacco, P. F. Spano and A. Tagliamonte*	743
Enkephalin receptors on dopaminergic neurones in rat striatum	*H. Pollard, C. Llorens-Cortes and J. C. Schwartz*	745
Blowfly ovipositor receptor neurone sensitive to monovalent cation concentration	*M. J. Rice*	747
Non-cholinergic excitatory transmission to intestinal smooth muscle cells	*T. Takewaki and O. Ohashi*	749
Calcium transients in frog slow muscle fibres	*R. Miledi, I. Parker and G. Schalow*	750
Induction of experimental allergic neuritis with a peptide from myelin P_2 basic protein	*S. W. Brostoff, S. Levit and J. M. Powers*	752
Myelination of mouse axons by Schwann cells transplanted from normal and abnormal human nerves	*A. J. Aguayo, J. Kasarjian, E. Skamene, P. Kongshavn and G. M. Bray*	753
Two mechanisms for positive inotropism of low-K Ringer solution in bullfrog atrium	*M. Goto, Y. Tsuda and A. Yatani*	755
Sarcomere shortening in striated muscle occurs in stepwise fashion	*G. H. Pollack, T. Iwazumi, H. E. D. J. Ter Keurs and E. F. Shibata*	757
Does electrophoresis of cockroach muscle proteins detect recognition molecules?	*N. M. Tyrer and K. A. Johnson*	759
Increased membrane fluidity precedes fusion of muscle cells	*J. Prives and M. Shinitzky*	761
Evidence for multispecificity of antibody molecules	*D. J. Cameron and B. F. Erlanger*	763
Internal motions of antibody molecules	*J. A. McCammon and M. Karplus*	765
Are late replicating regions in polytene chromosomes of *Drosophila* enriched by repeated nucleotide sequences?	*M. Evgen'ev, A. Levine and I. Gubenko*	766
Long-range attraction between red cells and a hydrocarbon surface	*D. Gingell, I. Todd and V. A. Parsegian*	767
The number of turns in globular proteins	*G. D. Rose and D. B. Wetlaufer*	769

BOOK REVIEWS

Pulsars (F. G. Smith)	*S. Jocelyn Bell Burnell*	771
Development in Mammals (Martin H. Johnson, editor)	*Anne McLaren*	771
Antinucleon–Nucleon Interactions (G. Eskpong and S. Nilsson, editors)	*P. I. P. Kalmus*	772
Platelets in Biology and Pathology (J. L. Gordon, editor)	*O. Behnke*	773
Parasitic Protozoa (J. P. Kreir, editor)	*W. H. R. Lumsden*	773
Problems in Vertebrate Evolution (S. M. Andrews, R. S. Miles and A. D. Walker, editors)	*Barry Cox*	774
Principles of Ultraviolet Photoelectron Spectroscopy (J. W. Rabalais)	*Neville Jonathan*	774

Obituaries

A. V. Hill	*B. Katz*	777
R. Kozlowski	*M. House*	778
George Cotzias	*M. D. Yahr*	779
C. F. Hickling	*M. Yonge*	780
J. A. Robbie	*G. I. Lumsden*	780

nature

7 July 1977

Getting at the truth about Dr Marshall

DR WALTER MARSHALL'S impending departure from his post as chief scientist at the UK Department of Energy (DEN), announced suddenly at the beginning of last week, raises certain obvious questions. Why? Why now? And what are the consequences? The matter is important. The storm accompanying the move has so far only amounted to a survivable squall rather than an intolerable hurricane for the minister who ordered it, Mr Anthony Wedgwood Benn. But it has rightly attracted more publicity than any similar move since the institution of chief scientist became a familiar part of the Whitehall scene. The reason is simple. It seems as though Marshall didn't fall; he was pushed—and almost certainly to make way for someone else.

The question of timing looks easy to answer, but isn't. True, Marshall had just completed the first round of reports on the exploitation of alternative energy sources; and important nuclear decisions are now coming up. Marshall had held two posts simultaneously—chief scientist at the DEN, for which he wasn't paid, and Deputy Chairman of the UK Atomic Energy Authority (UKAEA), with responsibility for its scientific and technical policy. So if he had to go from the former, this was the moment. As the blurb itself 'explained' at the time, Marshall would be resuming work in the UKAEA in view of those upcoming decisions and the UKAEA's 'significant role' in them.

It is not just that there is plenty of work still to be done in the non-nuclear field, in the execution of which Marshall is incidentally now a master, that has left people unsatisfied with this explanation. The various suggestions that Marshall was sacked, which Mr Benn has resented, have been based on the idea that when Marshall acted as Mr Benn's adviser rather than executor, he was 'pro-nuclear'; in other words, he was trying to push Mr Benn into taking early decisions on the choice of reactor issue and the fast breeder, and was generally giving unpalatable advice in an unpalatable manner.

If this argument has any credence at all, it demands refinement. For Marshall, formally speaking, was not Mr Benn's nuclear adviser. That was and is the statutory responsibility of the chairman of the UKAEA, Sir John Hill. Marshall would, of course, have given his view on nuclear issues if and when he was asked. And he had a good working relationship with Hill, his senior at the UKAEA, on responsibilities in this area. But if advice on the nuclear issues with which Mr Benn has become increasingly preoccupied is the reason for Marshall's departure, then *prima facie* it seems the wrong man is going.

Moreover, if the fact that Marshall is 'pro-nuclear' was now causing problems for Mr Benn, it had not caused him to worry in the past. It was Mr Benn who promoted Marshall 18 months ago to the Deputy Chairmanship of the UKAEA, even though Marshall was still to carry on being chief scientist. And it was Marshall who was reluctant to accept the promotion because of the conflict of interest, and who only did so after asking the minister to consider it again and after receiving assurances that he was satisfied.

It is possible that Mr Benn now regards this conflict of interest, much commented on in the past, as intolerable. Certainly Marshall must have felt the need for his departure to be made public immediately for his own personal integrity. But though decisions are due, nothing specific appears to have happened in the past 18 months to make Mr Benn change his mind. Neither is he likely to be grooming Marshall for the UKAEA chairmanship when Sir John Hill's appointment comes up for renewal later this year. Marshall may be only 45 with 20 years of public life left in him, but Hill is 56 and has 10 left himself. Hill's handling of his public relations may have been shocking—indeed appalling on the choice of reactor issue—but there is no sign that he is on his way out.

That being the case, putting Marshall back so precipitately into the UKAEA looks more like a humiliation than anything else. But what could Marshall have done? His record is almost impeccable. He has galvanised the department into action in the field of research and development, producing acclaimed reports on R&D strategy, alternative energy sources and combined heat and power. He has stimulated vitally important work in energy conservation and offshore technology. He has helped Mr Benn to put a young department thoroughly on the map in a way that Mr Benn may not even now appreciate.

Not only that. The consequences of his departure reveal an important paradox surrounding the whole affair. The move is a depressing setback for all those engaged in work on non-nuclear energy, outside as well as inside the department. The loss of morale could prove costly, not least for Mr Benn. At the same time, though, there is little reason to doubt that the affair will *not* prove a setback on the nuclear side, the area in which the real controversies have been raging and on which Marshall would only have given his view in an individual capacity as Mr Benn's personal adviser.

Now this could well have proved a problem, as many people have suggested. Marshall had arrived at particular views. He would have advised that, on the choice of reactor issue, Britain should switch to the pressurised water reactor and export as many as possible; that, on the fast breeder, one big reactor should be built with all deliberate speed; and that, on reprocessing, it should be done for environmental reasons and, by keeping recovered plutonium where it could be controlled, as an anti-proliferation policy.

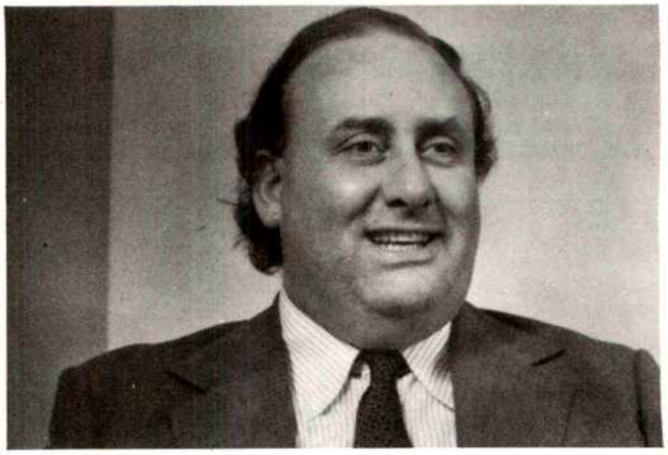
Some or all of this Mr Benn may have found unacceptable. But none of it seems to bear strongly on Marshall's departure, especially given that the UKAEA's position is more stridently 'pro-nuclear'. Only the manner in which such advice would have been given, and the mere fact that it was there impinging on decisions, seems directly relevant. Both of these appear to be strong possibilities.

Marshall and Benn are like chalk and cheese. Marshall will endeavour to appraise a problem and give a considered answer whatever its implications. Asked to do things, he gets them done—and with the department's thinking complete, a 'doer' is what it has needed and still demands. He will be a loss in that respect alone. Mr Benn is different. He must be happy with Marshall's record in executing R&D. But he would have found Marshall irritating to have around with nuclear matters such a big preoccupation. As an ideas man and a political animal to the core, moreover, he needs advisers to brainstorm with who are well-versed politically. And he needs them full-time. Marshall was no intellectual dilletante, and wasn't full-time. Benn is no scientist. And he may not want to get things done.

This other point, that delays are costly, is also important; Mr Benn's advisers have long been urging that decisions be taken. On the other hand, however, the nuclear pause, as Mr Benn calls it, is a bind for bureaucrats, who need goals and the work goals provide—and the nuclear field is a huge chunk of the department's landscape. The industry, too, wants decisions. Now it may well be that in favouring delay for the sake of a public debate Mr Benn has encouraged confrontation, and sought only to ride the back of a good issue for his own personal advancement. But to attribute motives of personal political convenience to actions that have helped to put a complacent industry properly on its toes may be unkind.

Suppose, then, that there may be a good deal in what Mr Benn himself says about now wanting an adviser who can range over the full spectrum of energy matters full-time. If Marshall's nuclear and non-nuclear experience doesn't equip him to do just that, and if it isn't enough simply to speak of Marshall as having suddenly become an unacceptable irritant, there must be a problem above and beyond the fact that to appoint Marshall to a full-time post as adviser would be a rebuff for Sir John Hill. Indeed, not to have made Marshall full-time adviser with a brief ranging over all fields is again a humiliation.

What could this other reason have been? The suddenness of the announcement suggests that the decision was not thought out. But it had apparently been brewing for some time. So perhaps it is better to look at things from the other side. Although it may really be better to announce decisions like this than have them leak out, not announcing a replacement, and saying that an official is to leave 'as soon as possible', is unusual. A presumption has to be made that a replacement will be anounced shortly.



Marshall: did he fall or was he pushed?

That, however, is difficult to do by recruiting from outside civil service ranks, and certainly cannot be done quietly. It is thus more likely that the post will be filled by someone already available. The obvious place to look is among the other chief scientists. Apparently Mr Benn wants another chief scientist as his adviser. And the one who has the necessary experience and who might fit Mr Benn's requirements—more than any other—is Sir Hermann Bondi, chief scientist at the Ministry of Defence. No one else is in sight, and Bondi, having done six years in his present post and being a greater believer in a varied diet, might well find the move attractive.

If that happens, plenty of people would welcome it, not least various people at the Ministry of Defence itself. So one would wonder whether the whole operation was perhaps set up before Marshall's departure was announced. If so, Mr Benn's advocacy of open goverment, sincere as it is, looks thinner as a consequence. More importantly, his experiences with Monty Finniston, Arthur Hawkins and now Walter Marshall suggest that he has a reputation for not being able to get on with his advisers. With such important decisions looming, that is the biggest worry of all. ☐

The concept of usefulness

Among other things, UK research councils are expected to support 'useful' research. In an article based on a lecture given at the Rutherford High Energy Laboratory, Sir Alec Merrison, Vice-Chancellor of Bristol University, comments

SCIENTISTS and society interact in many ways and the interaction is mediated by many agencies. The principal mediating agency in the UK is the government and in turn the government entrusts a great part of its mediating role to the research councils. Society makes demands of scientists and scientists make demands of society and in recent years these demands in both directions have become large.

In the past 15 or 20 years there has been a marked contrast between the mood of society in this country, very much determined by the sense of national failure, and the mood of science everywhere, which has been extremely buoyant. So government has looked to science (and technology) to be 'useful' and this was very much in the mind of the Labour government when it came to power in 1964 and again in the mind of the Tory government of 1970.

It is important to understand that the concept of usefulness is a decidedly limited one when applied to research, indeed it has to be applied with a good deal of taste. It is easy to identify certain research which will probably be useless in the economic sense, and just as easy to identify research which will certainly be useful. But these are limited areas and in between there is a vast body of research whose usefulness it is difficult, if not impossible, to quantify. Look at how much the world has been changed by the discovery 45 years ago of the neutron, and think how much it might be changed in the next 20 years or so by the discoveries of molecular and cell biology. But governments, and *a fortiori* ministers, do not last for 45 (or even 20) years and so, for this and other more compelling reasons, the time-scale is all-important in judging usefulness.

There have been two major revolutions in the past 15 years in the way research councils work: the one set in motion by the Trend Report of 1963 and the one flowing from the White Paper *A Framework for Government Research and Development* in 1972. It was on the Trend Report and the consequent Science and Technology Act of 1965 that our present framework of five research councils has been based. But one of Trend's recommendations, proposing the creation of an Industrial Research and Development Association, was inflated by the then new Labour Government into the now defunct Ministry of Technology. The aim of the 1965 Act was to bring some rationalisation and coherence to the civil research sponsored by public funds, and this it certainly did. But it was a recurrent theme through the 1960s that the research councils were not paying sufficient heed to the nation's needs, insofar as these were expressed by the government's spending departments. In 1970 this found expression in a bid by the Ministry of Agriculture to take over the Agricultural Research Council. This matter was examined by a committee of civil servants, chaired by Mr S. P. Osmond, who recommended that it should happen. Before agreeing, the then Secretary of State for Education and Science asked the Council for Scientific Policy, whose remit it was to advise her on all matters concerning the research councils, to advise her "on the most effective arrangements for organising and supporting pure and applied scientific research and postgraduate training". A small working party under the CSP Chairman, Sir Frederick Dainton, reported to her in May 1971 that its view was that the research councils should be left largely untouched but that the CSP itself should be reformed so that account could be taken of the views on policy held by government departments.

Just after this report was submitted, Lord Rothschild was appointed as first head of the Central Policy Review Staff and the government asked him to review the management of the whole of government research and development including that falling within the purview of the research councils. Lord Rothschild enunciated his now famous 'customer–contractor principle' which was to be applied to all research and development other than basic research. He recommended that in the case of three of the five research councils (Agriculture, Medicine and Natural Environment) there should be a large transfer of their funds to those departments which had an interest in their work. The departments would then use the research councils as their contractors to carry out work which they commissioned.

In the White Paper of July 1972 the government decided that the CSP should indeed be reformed, largely along the lines suggested by the Dainton Committee, and thus it became the Advisory Board for the Research Councils. They accepted too a modified form of Rothschild's customer–contractor principle and his proposal to transfer funds (roughly 50% from the ARC, 30% from NERC and 25% from the MRC). It is perhaps unnecessary to say that rather elaborate machinery has inevitably had to be set up to supervise the allocation of this transferred money.

There are three questions which can be asked in the case of each of these major reforms. Has it made any difference? Has it done society any good? Has it done scientists any good? Now is certainly the right time to ask these questions, since it was the Tory Government's intention when they published their 1972 White Paper that at least five years should elapse before any further substantial change should be contemplated. But in answering them it must be borne in mind that they are essentially questions which are not only difficult to answer, if indeed they can be answered, but also that any answers which can be offered must contain a good deal of subjective judgment.

Both reforms have certainly made a difference and I would imagine that this at least is an answer which would arouse little dissent. So far as the 1965 Act is concerned my own view is that both society and scientists have benefited. A reasonably flexible system has been created which at the same time is sufficiently cohesive that the 'Science Vote' is a good way to control the funds at the disposal of the research councils (not that they are ever enough!).

We are still rather close to the 1972 reforms, but five years later it is difficult to see that they have had any really substantial effect, and such effect that they have had seems to have been for the worse. They have certainly not done the scientists any good, but neither have they done them very much harm (with the possible exception of a good deal of nervousness on the part of scientists working in the more fundamental areas).

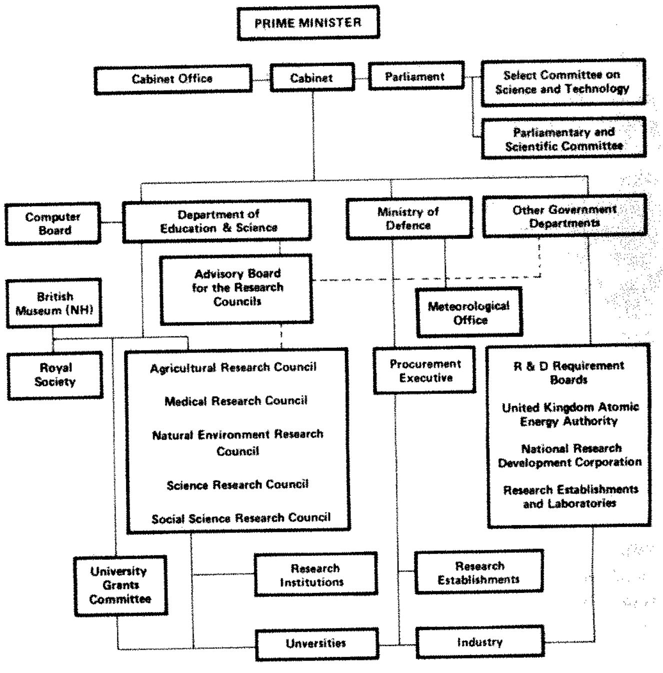
What has unquestionably happened is that an unnecessarily elaborate machine has been set up to supervise the spending of the money transferred from the research councils to government departments. This is certainly a loss to both scientists and society and should be reformed. It is wasteful of people's time and is yet another piece of inefficient bureaucracy.

Equally unquestionably, what has not happened is a re-direction of research to any conspicuous degree. One can ascribe this to the incorrigible waywardness of scientists or to the fact that they were not after all quite so insensitive to the needs of society as some people believed. My own view would be that it is largely the latter. At any rate, society is on balance the loser because of the 1972 reforms.

One of the weaknesses of the transferred money system is that once the control of a budget gets into the hands of a spending department then that budget will be at the hazard of all sorts of departmental pressures, few of which will have anything to do with whether the research which might be funded is good or bad. I can hardly believe that last year, when the DHSS decided not to spend nearly a million pounds of its transferred money, it took the decision simply on the grounds that it had come to the conclusion that it was not getting value for money.

So it is a good time to examine carefully the benefits and disadvantages of this way of government departments and research councils working together and, if it really is more trouble than it is worth, to scrap it. It would be good to look too at the whole conspectus of the government's support of 'useful' research and see whether the balance is right. Are we spending the right relative sums on aeronautical research and civil engineering, for example? But if any review is to be undertaken, on a narrow or broad front, I very much hope the reviewers bear in mind the fragile nature of the concept of usefulness in this field. □

UK Research Councils: Where they fit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OTA's stormy ride

Colin Norman in Washington examines the history and prospects of the Office of Technology Assessment

A SERIES of political upheavals has badly shaken Congress's Office of Technology Assessment (OTA) during the past few weeks, prompting speculation about the future of the fledgling agency and providing ample grist for the rumour mills of Washington's small corps of science policy watchers.

It began on 20 May, when Emilio Q. Daddario, OTA's founder and first Director, announced that he would resign to take an as yet unspecified new job. In his letter of resignation, Daddario said that he felt OTA was firmly established and noted that he had long expressed a desire to leave as soon as the office had reached that point.

Less than a week later Representative Marjorie Holt, a conservative Republican from Maryland, announced her resignation from OTA's Congressional Governing Board. The reason for her departure, she said loudly, was that OTA had been "taken over" by Senator Edward Kennedy, chairman of the board, and she felt that she could no longer make a contribution to OTA's affairs. Her grievances were promptly given a full airing by William Safire, a former speechwriter for Richard Nixon, who wrote a column in the *New York Times* charging that Kennedy had removed Daddario in order to install his own aide, Ellis Mottur, as director.

Then a third blow fell last week. The House of Representatives, acting on a recommendation of its Appropriations Committee, slashed the budget request for OTA next year from $9 million to $7.4 million and decreed that the office's staff level should be reduced to 100, thirty less than now.

This political crossfire is the culmination of a number of tensions which have been building up in OTA ever since it came into being three and a half years ago. The bloodletting that has resulted may ultimately be beneficial for the agency, but much will depend on who succeeds Daddario.

Difficult gestation

OTA's birth followed a long and difficult gestation period which began in the late 1960s. Congress was then beginning to grapple with a series of complex technological issues, such as whether the United States should proceed with construction of an SST and whether an anti-ballistic missile system should be deployed. The debates starkly underlined the fact that Congress lacked the resources to match the technical expertise of the executive branch on such complex matters. At that time Daddario was a Congressman from Connecticut and he held a key position as chairman of the science subcommittee of the former Committee on Science and Astronautics.

After holding a number of hearings with his subcommittee in the late 1960s, Daddario sponsored legislation to create an agency of the Congress to assist in the evaluation of technological matters and to provide an early warning system to pinpoint potential side effects and long term implications of projects and policies involving science and technology. Congress finally passed a bill establishing OTA in October 1972, though by that time Daddario had left Congress to run (unsuccessfully) for the governorship of Connecticut. Kennedy played a leading role in shepherding the bill through the Senate.

The bill specified that OTA would be run by a 13-member board, consisting of 6 Senators, 6 members of the House and the director of OTA. Republicans and Democrats would be equally represented on the board. In addition, the legislation established an independent advisory committee to provide policy advice to the board.

The legislation also specified that the chairmanship of the board should alternate every two years between Senators and House members. Kennedy was elected the first chairman. He was followed by Olin Teague, chairman of the House Committee on Science and Technology, who served during 1975 and 1976, and Kennedy resumed the chairmanship earlier this year. Daddario, meanwhile, was appointed director in 1973 for a six-year term.

OTA represented a major innovation in the Congressional process. It was designed to provide Congress with long-range analysis, act as a counterweight to the technological expertise of the executive branch, and function as an early warning system. It is very much a creature of the Congress, having to respond to the concerns of legislators and provide analyses of issues on a timescale which can effect the passage of particular bills. One OTA staff member last week likened OTA's establishment to an organ transplant: "We grafted it into Congress and waited to see if it would take", he said.

Daddario: just changing jobs?

The graft took slowly, and there have been occasional signs of rejection. First, it took a long time to get the initial studies under way because Congress was slow in appropriating money for OTA. There were also a number of internal problems in securing office space and assembling staff. But OTA began to crank out a huge volume of material. Some 46 studies have now been completed, and 34 more are in the works. Topics have ranged over a broad spectrum, including the bioequivalency of supposedly identical prescription drugs, policies to curb nuclear proliferation, the potential for small scale solar technology (see page 6), and a broad assessment of the impacts of offshore oil and gas production. OTA has also produced a number of critiques of specific administration policies, such as energy plans published by the Energy Research and Development Adminstration (ERDA), President Carter's energy policy, and the research and development strategy of the Environmental Protection Agency.

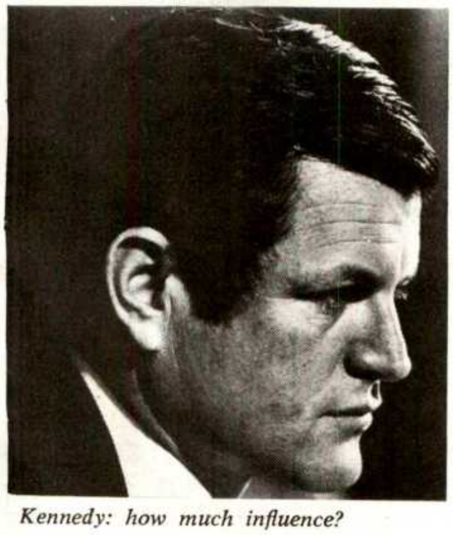
Though arrangements differ from one study to another, the general procedure is to establish an advisory committee of outside experts to help plan and monitor each project. The advisory panels are usually deliberately chosen to reflect diverse points of view, frequently containing members of public interest groups and businessmen. The studies themselves are usually carried out by OTA staff, sometimes with the help of outside contractors, under the guidance of the advisory panels and with overall management provided by a programme director.

Important criticisms

But OTA has run into a number of important criticisms during its short lifetime, and there have been occasional signs that Congress may reject this strange transplant. The grumbles have, however, concerned OTA's style of operation rather than the quality of its reports and analyses.

The first complaints came from OTA's advisory council, which grumbled that it was not being sufficiently consulted on matters of policy. Then, in June last year, the House Commission on Information and Facilities, an obscure Congressional unit headed by Representative Jack Brooks, charged that OTA's internal management was in a mess and that there was general confusion about the agency's mission. That blast was followed by criticism from Harold Brown, now Carter's Secretary of Defense, who resigned last July as chairman of OTA's advisory council and claimed in his letter of resignation that the office had become bogged down in responding to requests for relatively trivial policy studies to the detriment of its primary mission of providing an early warning system. Then last October, a House/Senate conference committee recommended that OTA's budget should be cut and suggested that the fledgling agency had yet to prove its worth to Congress. Finally, from outside OTA have come sporadic complaints that the office has been too cautious in its approach and that it has not really been performing technology assessment.

Asked to comment on some of those criticisms in an interview with *Nature* before he left office, Daddario acknowledged that, "Sure we have had our problems, but every institution in this town has had its problems". He pointed out that most of the criticism was directed at bureaucratic arrangements when OTA was still feeling its way and evolving its style of operation. It has taken three years to develop a sound process to handle the studies, Daddario said, and suggested that it could take at least a decade before the office is functioning smoothly with the full support of the Congress. Daddario noted that none of the criticism has been directed toward the quality or credibility of OTA's studies and it is that, after all, which will ultimately determine the office's influence.


Kennedy: how much influence?

It is indeed possible to point to a number of OTA analyses which had an impact on policy. The critiques of energy policy, for example, suggested that ERDA was paying too little attention to conservation, and the policy was subsequently changed. ERDA Administrator Robert Seamans acknowledged in a press conference early last year that OTA's analyses had played a significant role in shifting funds into conservation programmes in ERDA. The study on offshore oil and gas production has attracted attention and is praised for raising public awareness of many of the central issues involved in the Administration's plans. The drug bioequivalence study, which was the first published by OTA, played a major role in shaping federal policy for drug purchasing under government-financed health care schemes.

Though OTA staffers are quick to note such examples of the office's influence, there has so far been no coherent review of the quality of OTA's reports or the use to which they are put. Two such studies are, however, being planned. The first will be conducted this autumn by the House Committee on Science and Technology, and the second will be carried out by OTA's own advisory council. The latter study was initiated earlier this year by OTA's Congressional board, largely at the urging of Senator Kennedy. And that brings us back to the latest round of political upheavals at OTA.

Kennedy allegations

Allegations that Kennedy planned to take control of OTA were raised when the office was first established and Kennedy was elected chairman of its board. An article in the *Wall Street Journal* in 1973, for example, charged that Kennedy would use OTA as the springboard for his 1974 Presidential election campaign, and in the same vein Safire's recent column in the *New York Times* suggested that Kennedy has little influence on the Carter Administration so he is quietly taking control of OTA to build up his own power base.

Few people deny that Kennedy has a strong influence on OTA affairs. His supporters claim, however, that his influence derives from the fact that he is the most active and interested member of the board. His detractors claim that his influence derives from the fact that some of his own staff members work for OTA and that the chairman of OTA's advisory council, Jerome Weisner, is an old political ally of Kennedy's. It should be noted, however, that Kennedy is not alone in having his own staff working for OTA. Other Senate members of the board have at least one staff liaison with OTA, a fact which has drawn criticism from some other OTA staffers because the political appointees have divided loyalties.

Mrs Holt told *Nature* last week that she resigned from the board because she felt that it "was obvious that we were having no opportunity to make any input at all". On three recent issues Holt was outvoted on the board by Kennedy and his supporters. In each case, she was supported by Teague and the other two House Republicans. The first issue arose when the board refused to reappoint a Texas Instruments executive, Fred Bucy, to a second term on the advisory council. Kennedy opposed the reappointment because Bucy had attended too few meetings in his first term; Holt claimed that Kennedy simply didn't like Bucy's stand on some issues. Second, the board sanctioned a quick assessment of the data which led to the proposed ban on saccharin over Holt's objection that such a study wouldn't add anything to the debate. And third, Holt unsuccessfully resisted a move to have the advisory council conduct a study of OTA's operations.

Underlying the dispute is a significant political gulf between Holt, a conservative Republican, and Kennedy, a prominent liberal Democrat. OTA, in short, has become a battleground for partisan politics.

As for Safire's allegation that Kennedy had engineered Daddario's resignation to pave the way for installing his own candidate in the director's chair, Daddario insisted that his resignation stemmed from nothing more than a desire to change jobs. He said that he had originally planned to stay for only three years but stayed longer to see the solar study and the critique of Carter's energy policy through.

Whether or not these upheavals will leave lasting scars depends largely on who is chosen to succeed Daddario. Kennedy is taking scrupulous care to avoid suspicion that he is trying to influence the selection process. He has asked for nominations from scores of organisations, the advisory council will screen the candidates and the board will make the final selection. So far, the only announced candidate is Daniel V. Desimone, who has been deputy director for the past three and a half years. Ellis Mottur, who now heads a major OTA programme, has been nominated by several groups, but he told *Nature* that he has asked the council not to consider him because his close ties with Kennedy would be sure to encourage Kennedy's opponents.

The next few months are clearly going to be critical for OTA. ☐

Suit to measure

David Davies details a recent US court decision to settle a libel action involving scientists

A RECENT decision in the United States Courts of Appeals has overturned a libel judgment against the *New York Times* and an officer of the National Audubon Society. A New York jury had earlier ruled as libellous accusations in the *Times* that certain named scientists were paid by the pesticide industry to lie by misinterpreting bird-count figures; these figures might have been relevant to the question of DDT's effect on animal life. But the appeal court ruled that the reporting was privileged under the First Amendment.

The dispute arose out of the Audubon Society's report in *American Birds* on its 1971 Christmas Count. The editor, Robert Arbib, wrote a foreword warning that segments of the pesticide industry and certain paid "scientist-spokesmen" were misusing bird counts to prove that bird life is thriving. He continued that apparent increases had in most cases nothing to do with real population dynamics. They were the result of ever increasing numbers of birders, better access, better knowledge and more sophisticated identification. He concluded: "What we are seeing is a result of *not more birds, but more birders.* Any time you hear a 'scientist' say the opposite, you are in the presence of someone who is being paid to lie, or is parrotting something he knows little about".

A *Times* reporter, John Devlin, saw the article and asked Arbib to name some "paid liars". Arbib turned to Roland Clement, the society's Vice-President, for help; Clement provided a list of "consistent misinterpreters" of data, adding however, "We don't have any knowledge of anyone being a paid liar". Whether this qualification was actually passed by Arbib to Devlin was strongly disputed at the original trial.

Devlin next tried to get comments from the five named—J. Gordon Edwards, Thomas Jukes, Robert White-Stevens, Donald Spencer and Norman Borlaug. He reached White-Stevens, Jukes and Spencer, who hotly denied the charges—White-Stevens and Jukes sent copies of their publications. Devlin then wrote his story which appeared in the *Times* in August, 1972. It quoted the Arbib foreword extensively and added that Arbib had said in an interview that the men under attack included the five named. Devlin wrote that those he had reached "ridiculed the accusations as 'emotional', 'hysterical' and unfounded".

There was understandable alarm at the Audubon Society with the *Times* story, and Clement sent round an internal memo that "Arbib was trapped into listing people I have never called liars, though they may be". Clement also dispatched a letter to the *Times* over Arbib's signature saying "it is not simply that more birds are reported because there are more birders, but that we have more birders who are also better birders. Nor do we like to call people liars, but those who have most consistently misused our data [the five scientists were named] . . . have had time to learn from our patient explanations . . . ".

Jukes also wrote to the *Times,* claiming "we have reported these figures in birds per bird watcher". Neither letter was published. Jukes, White-Stevens and Edwards sued for libel and received a total of more than $60,000 in damages against the *Times* and Clement. Arbib was exonerated.

Libel actions by public figures in the United States (and the three plaintiffs were so ruled) can only succeed if it is shown that the libel was motivated by malice—that is, with knowledge that the charges were false, or with reckless disregard of whether they were false or not. The lower court apparently had no doubt that the charges were false and that insufficient care had gone

OTA's bright solar report

GIVEN relatively modest federal support, small solar energy devices could play a significant role in the United States energy picture within a decade, and their potential impact in many developing countries is even greater, according to a massive study published this week by the Congressional Office of Technology Assessment (OTA). Perhaps the most detailed report on solar technologies yet produced, OTA's 1,330-page document is likely to have a major impact on federal policy, coming as it does in the midst of Congressional debate on President Carter's energy proposals.

The report's central message is that the key factor governing the speed with which solar technologies capture the market is the relative cost of conventional energy resources. No major technological breakthroughs are needed for widespread use of solar heaters, and other technologies are also well advanced technically, the report says.

At present, solar heaters can compete economically with electrical heating systems in a limited number of situations, when the capital costs plus the lifetime fuel costs are taken into consideration. But the OTA report suggests that if electricity prices climb by a relatively modest 40% over the next quarter century, solar heaters will be economically attractive in most areas of the country by 1985. Moreover, if 20% of the capital cost of solar heaters could be offset against income tax, they would be competitive much sooner. As for non-electrical heating systems, the OTA report suggests that solar heaters will be cheaper than systems using synthetic gas, but will probably be more expensive than coal-fired systems at least during the next two decades.

The study is also optimistic about the potential for more exotic technologies, such as the direct conversion of solar energy to electricity by photovoltaic cells. Though such devices are now inordinately expensive, the OTA study suggests that the costs could be brought as low as 5–15 cents per kilowatt hour within a decade. That would still be well above present electricity prices, but it may be in line with electricity prices from new generators in the late 1980s. The key, again, is the extent of incentives such as tax breaks for both purchasers and manufacturers of photovoltaic equipment, but research breakthroughs will also be required to bring down the price of photovoltaic cells.

The OTA report breaks new ground in assessing the potential impact of a large solar industry on the labour market, and its findings are likely to enhance the political prospect for solar power. Widespread production and use of small solar units would create employment in such industries as sheet metal-working and light manufacturing which are now experiencing serious unemployment problems, OTA concluded.

Since the rate of introduction of solar technologies depends critically on the relative costs of competing energy sources, solar power is likely to be particularly attractive in countries where fuel costs are high and labour costs are low. Those conditions apply in most developing countries, and thus the OTA study concludes that the potential for solar power in the third world is great. Widespread use of solar technologies would carry the added advantage of reducing international competition for dwindling fossil fuels, and it would also reduce the incentives for developing countries to purchase nuclear plants, the report notes. It is thus in the United States' political and commercial interest to foster an international market for solar technology, the report points out, and the best way to stimulate such a market is for the United States to launch its own domestic solar energy programme.

Colin Norman

into investigating the allegations after the scientists had claimed they were baseless.

The appeal court in no way set aside the findings of the lower court that the accusations were defamatory. Indeed, it used the words "completely foundationless accusation of venality" to describe the charges. What it did rule, however, was that Devlin was insufficiently warned of the falsity of the charges by the scientists' responses, and so could not be accused of malice: "Mere denials, however vehement, are so commonplace . . . that they hardly alert the conscientious reporter to the likelihood of error". Nor, the court ruled, did the "largely irrelevant" mass of materials supplied provide a basis for warning of the falsity of the charges. There was not a "shred of evidence" that Devlin entertained serious doubts concerning the charges.

Further, the court held that even if Devlin had had such doubts, "We do not believe the press may be required under the First Amendment to suppress newsworthy statements merely because it has serious doubts regarding their truth". The public interest in full information required freedom to report charges; accordingly Devlin's article was held to be an "exemplar of fair and dispassionate reporting". Clement was also cleared because it was held that he had not known the *Times* would portray the five as paid liars when he supplied the list to Arbib.

The ways of the law sometimes puzzle scientists and perhaps the most puzzling aspect of this case is how a scientist, told he is to be called a paid liar, can adequately rebut the charge. The court ruled that simply calling the charges "emotional", "hysterical" and unfounded wouldn't even alert a reporter to falsity. Nor would sending him the scientist's stock-in-trade reprints, apparently do. It isn't clear that the appeals court looked at what was sent to the reporter, but they dubbed it "completely irrelevant to the libellous accusations".

It is also noteworthy that the one scientific issue got completely lost in the case—whether the bird statistics establish anything at all. Arbib's foreword claimed that bigger counts per birder arose from better techniques, but then implied that paid liars would simply fail to divide by the number of birders. Devlin did not even mention the question of improved techniques, implying much more strongly that certain scientists don't divide. But the scientists claim that they do and that the figures are still up. The question of better reporting *versus* bigger populations to explain these rises—the nub of the difference between the sides—was hardly advanced at all by this bloody court battle. ☐

ASBESTOS

Amplified evidence

Britain's Advisory Committee on Asbestos held hearings in London last week. Judy Redfearn reports

THAT there is a risk to health from asbestos cannot be denied; but among the general public and in the asbestos industry itself, there are conflicting views on how acceptable that risk is. In March 1976, the UK Health and Safety Commission set up the Advisory Committee on Asbestos to review the risk to different sectors of the public and to come up with recommendations on how asbestos should be used, if indeed it should continue to be used at all. Last week, the committee held three days of public hearings in London when it questioned some of those who had submitted evidence for its consideration.

At the heart of the debate was the acceptability of the asbestos dust levels which were recommended by the committee in an interim statement published in January. Occupational exposure to asbestos dust, that statement said, should be as low as possible, but should not exceed 0.2 fibres per cc when measured over a ten-minute period for crocidolite (blue asbestos, thought to be the most dangerous sort), and 2 fibres per cc averaged over a four-hour period for all other types of asbestos. But the safety of these levels depends on how accurately the incidence of asbestos-related disease can be predicted. As with other diseases caused by long term exposure to certain factors, such as the incidence of lung cancer in smokers, this is very difficult to assess.

The committee's recommended exposure levels were based on the results of a study carried out in 1966 by the British Occupational Hygiene Society (BOHS) on workers in an asbestos factory in Rochdale. The study concluded that an asbestos worker would have a 1% chance of developing an asbestos-related symptom if exposed to 2 fibres per cc for 50 years. A subsequent follow up study at the same factory, however, has come up with some different results.

Dr Julian Peto, of the Department of Health and Social Security's Cancer Epidemiology Unit at Oxford, was responsible for the later study. He told the committee that by the end of 1974 deaths from asbestos-related disease in those exposed for 25 years or more exceeded the total number of expected deaths by 30%. In 1966, the BOHS found nothing unexpected in the death rate. Peto's study also suggested that 5–10% of workers exposed for 50 years to concentrations of 1–2 fibres per cc of asbestos would be likely to die of asbestos-related disease. The discrepancy between the end result in the two studies, Peto suggested, could be due to the cumulative effect of asbestos exposure between 1966 and 1974 and the different way of sampling asbestos workers: Peto's study included all men who had been exposed to asbestos for 10 years or more between 1933 and 1972, whereas the BOHS study only included those still in employment there in 1966.

Furthermore, Peto told of evidence that chrysotile (white asbestos) could be as potent as crocidolite in causing asbestosis and pleural mesothelioma, an inoperable cancer of the membrane surrounding the lung. All in all he felt there was strong evidence that the present 2 fibres per cc level is inadequate but that there should be more research, especially into factors such as the effectiveness of pulmonary clearance below 2 fibres per cc.

But concern does not only centre on workers in asbestos factories. Asbestos is so widely used that most people at some time will have been exposed to it. Pressure is increasing to ban certain applications of asbestos such as asbestos paints and sprays and domestic use in general. The British Society for Social Responsibility in Science (BSSRS), using its evidence that cases of asbestosis and mesolthelioma have resulted from very short exposures to asbestos, is calling for a total ban on its use; it suggests that in the meantime personal protection for asbestos workers should be improved and safe substitutes should be introduced as soon as they are developed. But even a far more stringent standard poses problems. The recommendation from the Trades Union Congress of a 0.2 fibres per cc standard was thought technically impossible to achieve for most manufacturing processes, especially the dry processes which create a lot of dust and which are found in the asbestos textile industry.

The committee's task now is to digest the evidence gathered at the hearings and to call others forward to explain their case. No one yet knows when it will produce its report laying down recommendations for the future use of asbestos. There are provisions, however, for another interim report to be published if the evidence suggests that speedy changes are needed to the present ways of dealing with asbestos. ☐

CZECHOSLOVAKIA

In a sorry state

Vera Rich reports on recent evidence of harassment of dissident Czechoslovak scientists

CZECHOSLOVAK scientists whose "moral and political requirements" fail to meet the official standard are not permitted to publish their scientific work. This was revealed by a 270-page White Paper on Czechoslovakia published last week the International Committee for the Support of Charter 77.

The Order was first made known in a letter from the Czechoslovak Ministry of Education to the Charles University, Prague, in May 1972, and in spite of protests has never been revoked. Since recent government moves against the Charter 77 movements include widespread dismissals of known signatories, the order on publication has taken on renewed importance.

One man who visited London to launch the White Paper, Dr Zdenek Mlynar, was dismissed from the Entomological Department of the National Museum for being a member by the International Committee for the campaign known as Charter 77". Now in exile, he says that with the new waves of dismissals of the known Chartists from the Party and from professional employment, "Prague today is full of highly educated men sweeping the streets and stoking boilers".

These dismissals differ in one respect from those of the post-Dubcek repressions of 1970. Then it was primarily the social sciences which were hit—offending natural scientists were demoted but normally remained in their field of research. Today, however, the campaign against the Chartists makes no distinction between natural or social scientists; all are equally liable to dismissal, under the regulations on political attitudes.

These regulations, quoted in the white paper, actually antedate the Charter. The Directives of the Secretary General of the Academy of Sciences of July 1976 noted that "All creative and economic-technical employees shall have to be qualified in terms of politicals maturity . . ."; and all leading "scientific workers" of the Academy were requested to complete a questionnaire concerning their political activities and Party status, and also that of their close relatives.

Following the appearance of the Charter, there was a wave of summary dismissals of known Chartists, including Dr Mlynar himself, from academic posts without the customary period of notice; it was "not possible" for the Chartists to remain during the normal termination period "without jeopardising the orderly fulfilment of the organisation's tasks." Under the 1972 regulations on publication, scientists thus dismissed not only lose their posts; they are unable to publish work in hand, or undertake such fringe activities as translation.

When the restrictions first became known, Dr Frantisek Janouch, in a letter of protest to the Minister of Education, Josef Havlin, pointed out that they would inhibit the dissemination of "valuable information on new findings" solely because their authors' "moral-political profile does not comply with a certain set of curious criteria which have never been publicly announced". In the case of the Chartists, the criteria are even more curious because the Charter and its subsequent documents make no demands which are not already stipulated either in the Czech constitution or in the international human rights agreements to which Czechoslovakia is a signatory.

Whatever the criteria, however, the dismissal of scientists and the ban on publication are a considerable threat to Czechoslovak science. It is already difficult, said Dr Mlynar, to function adequately as a scientist in Czechoslovakia: personal international contacts are now only 10% of what they were in 1968; foreign currency restrictions have brought about a general drop in the standard of equipment and in the accessibility of foreign journals.

Now there is also a threat to the next generation of scientists. A large collection of documents in the white paper presents unequivocal evidence that parents' political attitudes are a prime factor in deciding university admissions. Again, this trend antedates the Charter; Document 4 of the Chartist movement (23 January 1977) specifically calls for an independent inquiry into this practice. This demand has not been met. □

WEST GERMANY

Saving it

The West German government has plans to encourage energy saving. Werner Gries explains how

CONDITIONED by the oil crisis the Federal Republic of Germany has now moved energy-saving to the foreground of its energy policy. It has put special emphasis on reducing energy losses in electricity generation, saving energy in heating supply, and saving energy used by traffic.

On the domestic front low interest loans have been allocated to finance energy-saving measures. Definitive regulations about heat insulation for housing construction have been laid down in energy saving legislation and new buildings will have to be better insulated in future. Firing installations in residential buildings will also have to be made more efficient. This will increase the cost of building houses by approximately 3%.

Industry is to be granted 7.5% of its expenditure on energy-saving investments and special investment credits are already available. In the public sector energy-saving measures are being considered in building plans and public education in energy saving is being intensified. In research, prototype projects for the efficient use of energy are being supported directly by the Research Ministry; over the past three years about DM500 million have been made available for this purpose.

The government is at present working out a detailed programme. At the heart of further domestic measures are investments to improve heat insulation and installations for heating supply. Heat pumps and solar collectors are also to be promoted through state allowances. The tenancy law is being altered to encourage energy saving. The government also wants to modify electricity tariffs and possibly make it a duty to mark the energy consumption on household appliances.

The focus in industry is on the coupling of electricity and heat production. An investment of DM2,400 million is to be spent on district heating up to 1980. Thought is also being given to taxing cars with diesel engines less than those with petrol engines and new driving systems (methanol cars and electric cars for example) are being encouraged through tax incentives. Most of the government measures, however, will only complement private initiatives and interventions will be limited. □

SWEDEN

● The National Environment Protection Board has proposed to the government that, from 1 January 1979, no aerosol pack containing halocarbons as a propellant should be made in Sweden or imported without permission from the Board's Product Control Division.

The Swedish initiative follows American research into aerosol packs and their effect on the ozone layer—an area in which the Swedes have not done any independent research. The present proposal was stimulated by last May's joint recommendation by America's Food and Drug Administration, Environmental Protection Agency and Consumer Product Safety Commission that would forbid the manufacture of halocarbons or of sprays containing them and the sale of both by April 1979.

The American recommendation exempts aerosols for certain essential uses, mainly medical (sprays for asthmatics, for example), and envisages other uses deemed essential in the future being added to the list of exemptions. The Product Control Division chemist who wrote the Swedish proposal, Ingrid Jedvall, sees much the same sort of situation emerging in Sweden, with certain aerosols being given permission to be sold. As an administrator she prefers her proposal to the American one because if all decisions about particular aerosols are taken within the division, no publication and updating of exemptions will be necessary. It could also be argued that the public should be informed which aerosols are being sold at any time. Critics will no doubt label the proposal as typical of a Swedish trend, which they see as government by bureaucrats whose tenure does not depend on responsibility to the people.

Sweden does not manufacture halocarbons, and uses less than 1% of the world's consumption. In 1975, the country used about 5,200 tons of halocarbons, but this amount has decreased to the extent that the 1977 total is expected to be only about 1,200 tons. The 1975 amount was used in the following proportions: cosmetics 65%, household products 6%, medical and veterinary 3%, colour, waxes and polishers 9%, insecticides 4%, products used in industry 12%, and other uses 1%. In the same year, 29 million packs were sold, an average of about 3.5 for every inhabitant.

The decreased use of halocarbons is partly due to changes in production introduced successively since 1973 by the Aerosol Packing Co., which sells 75% of aerosol cans in Sweden. It has changed from halocarbons to propane and butane and, as a company spokesman pointed out, will not be at all dismayed if halocarbons are regulated. To offset the fire risk present during manufacture under the new régime, the company has computerised the system so that the cans are monitored and the whole system can shut off automatically if concentrations of the gases reach the danger zone.

● Sweden's first wind power station was recently opened on the coast north of Stockholm, at Älvkarleby. Its 18-metre-wide vane, which rotates about an axis perched on top of a tower 25 metres high, generates between 50 and 63 kilowatts. Ungainly though it looks, its computer—which automatically adjusts the angle of the vane to the wind—should provide the state Committee for Energy Research with useful data about the way its design stands up to stress and vibrations under different wind conditions. It is, says one committee member, Sven Hugosson, a sort of laboratory bench unit whose performance will determine whether it will be worth building others of the same type. The first evaluations of the way it is going will be made this summer.

Whatever the results, they will not influence the next phase of the Committee's wind programme, due to begin in August. Three full-size wind power stations, each generating 2 megawatts but differing in design, will be located in various parts of the country with the help of a survey done by the Meteorological Institute describing wind conditions around the coasts. Their performances will determine which designs will be built in future. At the opening of the Älvkarleby station the Energy Minister, Olof Johansson, looked forward to the turn of the century, when he envisaged wind energy providing between 4,000 and 10,000 megawatts.

Sven Hugosson says he does not doubt the technical feasibility of wind power for Sweden, but that very little money has so far been appropriated for experimentation. He does not think it could replace any other source of energy, but is certain it could play a part—especially in conjunction with hydro-power—in providing total energy needs. It does have, however, some surprising hazards: chunks of ice may be flung off the vane in winter and scattered like mini-missiles.

Wendy Barnaby

IN BRIEF

JET: still waiting

Some intensive lobbying is expected throughout Europe over the next couple of weeks in the run-up to the EEC Council of Foreign Ministers in Brussels on 25–26 June, when a decision on the siting of the Joint European Torus (JET) fusion project is now due. The subject came up at the meeting of European heads of government in London last week. Reports of a West German effort to secure a greater UK contribution to the Community budget in return for the project going to Culham instead of Garching were not in the event borne out. The leaders failed to agree and the matter was passed over to the Foreign Ministers for them to decide in a make-or-break meeting.

At a press conference after the meeting, the UK Prime Minister, James Callaghan, said he doubted whether any individual country could carry on on its own if a solution satisfactory to everybody wasn't reached. But with such an excellent team at Culham, he said, "It would be my desire to see whether we could work out bilateral, trilateral, quadrilateral or multilateral agreements with any group of countries that will keep the project in Europe, even if it wasn't a European project in the technical sense".

World climate conference

At its 29th session in Geneva last month the executive committee of the World Meteorological Organisation (WMO) decided to convene in 1979 what it calls a high-level scientific and technical conference, called the World Climate Conference, gathering together meteorologists and experts on the effects of climate on economies.

The timetable of the first global experiment of the Global Atmospheric Research Programme (GARP) was approved. Gathering of atmospheric and oceanographic data for research will be planned this year and observations will be carried out from December 1978 to November 1979, especially during January–February and May–June 1979. The aim of the experiment will be to improve meteorological forecasts and to obtain a better understanding of climate.

Energy balance urged

Governmental machinery for striking the right balance between different energy sources, bearing in mind not only availability but the differing community and environmental costs, is "entirely lacking', says Sir Peter Kent in a letter to *The Times* this week. Sir Peter, chairman of the UK Natural

Environment Research Council (NERC), would like to see some interdepartmental organisation established —perhaps within the Cabinet Office— "to demonstrate that the energy problem is regarded as transcending departmental boundaries". Factual data are, he claims, entirely inadequate at present for the general public, and the conventional public inquiry is too narrow in its scope to allow for truly informed decisions. In his opinion, the research council system could be of value in providing independent and authoritative data for such enquiries.

SRC appointment

The UK Science Research Council last week announced the appointment of Mr A. M. Adye (49) as its first Director of Marine Technology. He became Director on 2 May and is on secondment from British Petroleum Ltd.

The SRC's Marine Technology programme itself is new: it was announced only last year. Its aim is to involve academic institutions in all aspects of marine technology and to encourage postgraduate engineers to take up opportunities in the exploitation of the sea and sea-bed resources. Eventually, the Marine Technology Directorate hopes to promote the development of centres of expertise in marine technology in universities and polytechnics.

A Management Committee of six members will determine the outline of the programme, and three assessors from the Department of Energy, Department of Industry and Ministry of Defence will also be involved.

Energy body announced

The UK Secretary for Energy, Mr Benn, announced last week the composition of the Energy Commission which is being set up to advise the government on the development of an energy strategy for Britain.

Seven members of the 23-man Commission will be the chairmen of the National Coal Board, the Electricity Council, the British Gas Corporation, the United Kingdom Atomic Energy Authority, the British National Oil Corporation, the South of Scotland Electricity Board and the Petroleum Industry Advisory Committee. Seven others will be drawn from the TUC Fuel and Power Industries Committee, and the remaining seven, whose names will be announced shortly, will represent other industries and consumer interests. The Minister of State for the Scottish Office will also be a member and the chair will be taken by the Secretary of State for Energy.

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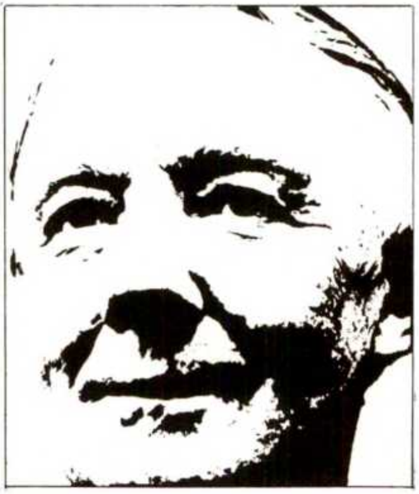

KENNETH MELLANBY

As an entomologist I spent many years killing insects. During the 1939–45 war I played some part in controlling medical pests in Britain, Africa and South East Asia, and was involved in operations where large amounts of toxic insecticides were widely and effectively distributed. I have also had some experience with agricultural pests, and nuisance problems such as swarms of gnats breeding in sewage plants. I was not the friend of the insect world, and so should not have been surprised when I suffered from its attentions.

As an undergraduate, I took part in an expedition to the Canadian Arctic. We made botanical collections and anthropological measurements of Eskimos, but it was the biting insects which impressed me most and which probably diverted my career from either medicine or botany. The most effective repellant then available was citronella oil, but liberal applications of it, a thick veil and army puttees did not prevent me from receiving thousands of mosquito bites. There are probably drops of blood from my wounds on the dried plants which were eventually sent to the herbarium at Kew in England.

Another revolting creature which has added to my sufferings is the bedbug. As a young research student at the London School of Hygiene and Tropical Medicine, I lived in a seedy flat in Soho which we kept reasonably bug free, but on hot summer nights these insects, moving out of less well maintained property, crawled in at the windows until these were surrounded with sticky bands of the grease used to stop pests climbing up fruit trees. I met bedbugs in every country I visited—in wicker chairs in Ceylon, where they bit the backs of my bare knees, in a rather grand hotel in Calcutta where I caught over 200 in an hour and sent them to the manager with my compliments, and even in Stockholm, Sweden, in a cheap hotel in a picturesque part of the old city, now replaced by hideous modern buildings.

Then about ten years ago I lectured to the British Association for the Advancement of Science on 'Insecticides'. Halfway through my talk I felt a painful jab in my scalp; I had been stung by a wasp. With true British *savoire faire* I remarked, casually, to my audience, "I've been stung by a wasp", and continued my lecture. But somehow the press got to know, and next morning the national newspapers contained the story with the headline: 'Insect world strikes back'.

And now it has struck again. This summer my seventeenth century farmhouse in rural England has developed a massive attack of Death Watch Beetle. I was laid up in bed for a few days, and on a warm afternoon towards the end of May I observed hundreds of these beetles emerge from what the house agents would call the 'wealth of old oak beams'. They crawled actively in all directions, and, when the temperature was above 22 °C, many flew vigorously and dashed themselves against the window panes. It was only the bedroom I occupied that produced swarms of beetles; the rest of the house yielded very few. The attack was obviously aimed at me, personally.

In some ways this is a bit unfair. For the last few years I have mended my ways, and have been mainly involved in wildlife conservation. I have helped to control the use of the more dangerous insecticides, as part of an attempt to safeguard the environment. I have banned pesticides from my garden, and have relied on the inefficient help of beneficial insects. But the Death Watch Beetle has gone too far. Now I have willingly agreed to have my house impregnated with one of the organochlorine insecticides. This is being done with care, and I hope that little will escape to harm the birds and insects in the garden. But this is war to the death, and nature must take its chance. I am even reconsidering my policy for pest control in our garden.

news and views

Population genetics and cultural inheritance

from Robert M. May

Many of the central questions of our time, both at the barricades and in the ivory tower, turn upon the relative contribution made by genes and by environment to the behaviour of individuals.

Disentangling these factors in any specific situation is often made forbiddingly difficult by the fact that the contribution of nature depends on nurture, and that of nurture depends on nature. Medawar (*New York Review of Books* **24** (1), 13; 1977) gives an illuminating example. The little brackish water shrimp *Gammarus chevreuxi* begins life with red eyes, but usually ends up with black eyes. This blackness arises from the deposition of melanin in the eye, a process which depends both upon the genetic predisposition to form and deposit melanin, and upon the temperature and other features of the environment. Particular genotypes can be chosen such that the eye colour is determined wholly environmentally (black at relatively high temperature, red at low); conversely, it is possible to choose temperatures such that the eye colour is inherited in perfect Mendelian fashion, like the classic peas. The simple question 'what percentage of colouration of the eye is due to heredity?' is meaningless. Of course, for cases like this shrimp, or for most plant and animal breeding, the relative contributions of nature and nurture can be sorted out by careful programmes of study and experiment. For human societies, the relative roles of heredity and culture (or, to use a word newly minted by Dawkins in *The Selfish Gene*, Oxford, 1976, of genes and 'memes'), are manifestly more difficult to disentangle. Furthermore, as Medawar has lamented (*op. cit.*): "The subject is bedeviled more than any other by the tendency of disputants to spring into political postures which allow them no freedom of movement. Thus it is a canon of high tory philosophy that a man's *breeding*—his genetic makeup—determines absolutely his abilities, his destiny, and his deserts; and it is no less characteristic of Marxism that, men being born equal, a man is what his environment and his upbringing make of him. The former belief lies at the root of racism, fascism, and all other attempts to 'make nature herself an accomplice in the crime of political inequality' (Condorcet) and the latter founders on the fallacy of human genetic equality ('A strange belief,' said J. B. S. Haldane—a long-time member of the CP)."

It would be nice if we could turn to theoretical population genetics for help. The mainstream of this subject, however, is devoted to increasingly elaborate studies of the way gene frequencies behave under the influences of mutation, migration, drift and abstract selective forces; the selection is rarely tied to concrete environmental realities.

Models for cultural inheritance

Of great interest, therefore, is the recent work of Feldman and Cavalli-Sforza (*Theor. Pop. Biol.* **4**, 42, 1973; *Amer. J. hum. Genet.* **25**, 618, 1973; *Annls hum. Biol.* **2**, 215; 1975; see also Karlin & Carmelli *Proc. natn. Acad. Sci. U.S.A.* **71**, 4727, 1974; and Lewontin *Am. J. hum. Genet.* **26**, 400, 1974). Although still in the formative stages, this work treats models for cultural inheritance, and seeks to show how traits may evolve under combined genetic and phenotypic transmission.

The most recent of these studies (Feldman & Cavalli-Sforza *Theor. Pop. Biol.* **9**, 238; 1976) begins with the familiar artifice of a trait that depends on one locus with two alleles, A and a, thus leading to the three genotypes AA, Aa, aa. Now comes the twist: adult individuals are characterised not only by their genotype, but also by whether or not they possess a certain unspecified skill. There are thus six possible phenotypes, the skilled ones $\overline{AA}$, $\overline{Aa}$, $\overline{aa}$ and the unskilled ones AA, Aa, aa. For the initial study, one parent, who may or may not be skilled, is arbitrarily defined as the "teaching parent". In general, the 'educability' of the offspring will depend on its genotype so that the probability that a given individual will grow up to be skilled now depends both on the genotype of the individual (his nature) and on whether the teaching parent is skilled (his nurture). Finally, it is supposed that selection occurs; the six different phenotypes will have relative reproductive success rates that depend on their genotypes and on their acquisition of the skill.

The assumption is now made (more for convenience than from conviction) that mating occurs at random with respect to skill and genotype. Equations may then be constructed relating the frequency of the genes A and a, and the proportions of skilled compared with unskilled individuals for each of the three genotypes, between successive generations. In their most general form, these equations are highly complicated: I shall return to this below.

Feldman and Cavalli-Sforza extract interesting results by making various simplifying assumptions. In the first case they assume that the selection coefficients depend only on whether the individual is skilled or not; the relative reproductive success of any skilled to any unskilled phenotype is $1+s:1$, independent of the genotype. The genotype is of course still important in determining the probability that the phenotype will be skilled (by influencing the 'educability' of the individual), but the selection coefficients do not depend on the genotype as such. On the assumption that unskilled teaching parents never raise skilled offspring, it can be shown that the maintenance of genetic polymorphism (both A and a present at equilibrium) requires not only that heterozygotes (Aa) be more receptive to the skill of the parent

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than are the homozygotes (AA and aa), but also that this 'overdominance' in heterozygotes' learning ability must exceed some threshold that depends on the selection parameter s. The authors give as a numerical example the case where a skilled teaching parent raises 70% of its AA or aa offspring to be skilled compared with 80% of the Aa offspring; polymorphism is maintained only if the selective margin in favour of skill exceeds 1/3 (that is, $s>1/3$; $1+s:1>4:3$). This is in contrast with conventional population genetics, where heterozygote superiority ensures stable polymorphism.

In the second case, Feldman and Cavalli-Sforza make a further simplification and assume that all offspring of skilled teaching parents are skilled. There is now completely accurate copying of the teaching parent (skilled or unskilled) by all offspring, irrespective of genotype. Nurture is all-important; nature is irrelevant. Obviously, the final state is for all the population to be skilled (providing the skill is advantageous, $s>0$). Less obviously, there is no unique value for the frequency of the genes A and a; rather, there is a neutral equilibrium, with the gene frequency depending on the initial distribution of the skill effects. Even though there are no genetically based selection differences, substantial changes in gene frequency can occur over time if there are initial differences in the distribution of the skill among the genotypes. As an example, the authors assume that the alleles A and a are initially equally common, and that the initial fractions of the genotypes AA, Aa and aa that are skilled are 90%, 50% and 10% respectively: this system will eventually settle to have the frequency of the alleles A and a around 65% and 35% respectively, corresponding to a 30% change in gene frequency. To the unsophisticated observer, it might appear that the 'superior' gene A has asserted itself; in fact, this relatively large gene frequency change is due to a kind of founder effect in a situation where all genotypes are equivalent.

Up to this point, it has been assumed that the probability of acquiring the skill (with consequent selective advantage s) depends on one 'teaching' parent. The authors next consider the case where both parents affect the outcome. To keep things manageable, the genotype of the offspring is assumed to be irrelevant; the probability that an individual will grow up to be skilled depends only on the phenotype of both parents. In these cases, the gene frequencies of A and a approach some neutral equilibrium, determined by initial values. In contrast, the phenotypic proportions of skilled and unskilled individuals converge to equilibrium values that depend on s and on the probabilities for given parental combinations to produce skilled offspring. Depending on the relations between these parameters, there may be a unique stable state, or two alternate stable states, or even (in rather extreme circumstances) stable cycles in the proportions of skilled phenotypes.

Disadvantageous 'cultural' traits: the 'kuru model'

In the models where acquisition of the skill depends on both parents, it is interesting to consider the case where the 'skill' confers a disadvantage ($s<0$). Such could be the case where an infectious disease or other such disadvantageous trait is transmitted from parent to offspring. Feldman and Cavalli-Sforza show that 'a strongly disadvantageous custom can spread through a group provided there is enough pressure on the members of the group to conform to the custom' (page 257). The authors refer to this as 'the kuru model', after the degenerative brain disease found among the South Fore linguistic group in the Eastern Highlands of New Guinea, and thought to derive from a slow virus (similar to that producing the rare Creutzfeldt–Jakob disease) transmitted by the custom of eating or sniffing the brain of dead relatives. The authors add that 'in these cases it would be of considerable interest to introduce the added feature of finite population size in order to

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New shocks in northeastern Alaska

from Peter J. Smith

EARTH scientists have often displayed a tendency to interpret a lack of data prematurely in terms of simplicity, presumably through an over-literal application of Occam's Razor. Only a few years ago, for example, it was not uncommon to find the upper mantle being described without qualification as physically homogeneous; and yet as more and more data have accumulated the illusion of homogeneity has receded. This happens to be one of the classic examples of oversimplification through ignorance, but there are many hundreds of others of various degrees of importance, one of which has now been revealed for what it is by Gedney *et al.* (*Geophys. Res. Lett.* **4**, 175; 1977).

This new case, which concerns the seismicity of northeastern Alaska, is perhaps of minor importance scientifically but warrants attention because of its political overtones. In April 1976 Meyers (*NOAA Technical Memorandum EDS NGSDG-1*) could find mention of only 10 earthquakes having occurred in Alaska north of 68°N throughout the whole of recorded history, thereby apparently confirming the widely held view that northeastern Alaska is relatively aseismic. The fact is, however, that the first seismograph station in Alaska was not constructed until 1935, and from then until 1966 the station closest to the northeastern corner of the state lay several hundred kilometres away near Fairbanks. Moreover, even as recently as 1975 there was no station closer than Fort Yukon on the Arctic Circle. In short, the detection threshold for earthquakes in northeastern Alaska has traditionally been high.

But all that changed during 1975–1976 when Gedney and his colleagues installed nine new seismographs in the Brooks Range. This sounds easier than it was, for access to much of this area is by helicopter only. Maintenance during the winter months is therefore impossible; and the recording equipment has been usable for only about half of the time. Nevertheless, during 1976 no fewer than 69 earthquakes in the magnitude range 1–4 were recorded and located (44 of them north of 68°N) and many others were detected but could not be precisely located because of low shock magnitude or equipment failure. In other words, the result of a drastic reduction in the threshold detection limit (from about magnitude 4 to magnitude 1) has been to erase the mistaken impression that the northeastern corner of Alaska has a very low seismicity.

This may not be the first time that an area has been shown to be more seismic than previously supposed. The point about northeastern Alaska, however, is that it is the site of the northern section of the oil pipeline and of the Alaskan section of the proposed natural gas pipeline through Canada. The northern part of the oil pipeline has in fact been designed to withstand shocks in excess of magnitude 4. But discoveries such as that by Gedney *et al.* are bound to strengthen the environmentalists' insistence that any new pipeline projects should be deferred until the data available are adequate to rule out even nastier surprises.

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consider the problem ecologically, as one of potential extinction or survival of the group'. Indeed, kuru probably speaks more to this *caveat* than to the basic model, as the disease is now thought to have appeared around 1910 (Burnett & White *Natural History of Infectious Disease,* Cambridge, 1971; Marsh in *Slow Virus Diseases of Animals and Man,* ed. Kimberlin, North–Holland, 1976), and it is hard to see that a group could long sustain a custom with such damaging consequences; during the peak incidence of the disease, in the 1950s, 1% of the Fore population would die of kuru each year, with the prevalence of active kuru reaching 5–10% of the inhabitants of certain tribes (Marsh *op. cit.*).

The authors also show that if any strongly disadvantageous trait can be learned outside the family environment, then a low frequency of the trait may be maintained, in a manner completely analogous to the classical mutation–selection balance of population genetics.

Formidable mathematical difficulties stand in the way of a more full understanding of the interplay between cultural and biological evolutionary processes. As noted by the above authors, and, in a related context, by Oster, Ipaktchi and Rocklin (*Theor. Pop. Biol.* **10,** 365; 1976), by Slatkin (*Proc. natn. Acad. Sci. U.S.A.* **66,** 87; 1970) and by Lande (*Genet. Res. Camb.* **26,** 221; 1975), the general equations relating the gene frequencies in successive generations are not only non-linear, but also involve frequencies from yet earlier generations. The resulting dynamics can have alternate stable points, or a variety of stable cycles, or even apparently chaotic fluctuations that are in many ways indistinguishable from random noise (see the reviews by May *Nature* **261,** 459; 1976; and by May & Oster, *Am. Nat.* **110,** 573; 1976). Nevertheless I believe that the incorporation of cultural inheritance into the quantitative theory of population genetics, as begun by Feldman, Cavalli-Sforza and others, is likely to open exciting new areas. □

Ancient distribution of lime trees in Britain

from Peter D. Moore

Two species of lime tree, *Tilia cordata* and *T. platyphyllos,* together with their hybrid, are native to the British Isles. They are warmth-demanding trees and reach their climatic limits in Britain, neither species occurring naturally in areas with a July mean temperature less than 15 °C. Pigott (*J. Ecol.* **57,** 491; 1969) considers that woodlands containing these species have often been undisturbed over long periods of time, for they frequently contain a characteristic assemblage of plant species which is lost in disturbed woodlands. Interference by man in such woodlands, he claims, often leads to the replacement of lime by ash, elm and sycamore. Such a view was given support by the palynological work of Turner (*New Phytol.* **61,** 328; 1962), who demonstrated the coincidence of the decline in *Tilia* pollen, often in Iron Age times, with evidence of human activity.

Within the British Isles, *Tilia platyphyllos* is the more restricted in its distribution, and both species are generally associated with base rich soils of mull humus type. *T. cordata,* however, does occur on acid, well-drained soils in eastern Europe in association with pine and spruce (Pigott *Phil. Trans. R. Soc.* **270,** 151; 1975).

The past history of the genus in Britain is rather confused for a number of reasons. Identification to specific level has only been undertaken as a routine process in recent years, since Andrew (*New Phytol.* **70,** 683; 1971) showed that exine patterning provides a reliable guide. Subsequent counts in which separation has been effected, such as those at Holme Fen by Godwin and Vishnu-Mittre (*Phil. Trans. R. Soc. Lond.* **270,** 561; 1975) show a strong predominance of *T. cordata* throughout the post-glacial, which corresponds with present-day relative abundance of the two species. Most other published and unpublished data from other parts of Britain confirm this.

Before human interference with woodland became a major factor in the history of our vegetation, say 5,000 years ago, the pollen of lime was distinctly more abundant in southern and eastern England. Birks, Deacon and Peglar (*Proc. R. Soc. Lond.* **B 189,** 87; 1975) have mapped *Tilia* pollen frequencies at that time and show only East Anglia, the east Midlands and the south-eastern extremity of Britain with values of 11–15% of tree pollen. There were times, however, during the height of forest development in this country when lime must have been locally abundant. In East Anglia at Holme Fen, values up to 50% arboreal pollen were recorded, and up to 30% at Old Buckenham Mere (Godwin *New Phytol.* **67,** 95; 1968).

Being insect pollinated, *Tilia* is probably under-represented in aerial pollen fallout, although according to Godwin (*History of the British Flora,* Cambridge University Press; 1975) it produces large quantities of pollen. It is difficult, therefore, to interpret such values in terms of lime abundance in the contemporary woodlands.

It is interesting to note that high *Tilia* proportions have also been recently reported from areas on Cretaceous and Tertiary sands and gravels in the south east. For example, unpublished analyses of J. A. Webb on Hothfield Common, Kent (Lower Greensand) have *Tilia* cf. *cordata* pollen values up to 40% of arboreal pollen. On the northern side of the North Downs from this site, at Wingham, near Canterbury, a value of 20% was obtained by Godwin (*Veröff. geobot. Inst. Zürich* **37,** 83; 1962). In this analyses of Wealden soils, however, Dimbleby has failed to record high *Tilia* pollen levels (see for example, *Proc. Prehist. Soc.* **26,** 246; 1960; **31,** 85; 1965; *Grana Palynol.* **4,** 140; 1963, all sites on the Wealden Lower Greensand). It is interesting to note in passing that one of Dimbleby's sites, Addington in Kent, was the location of the first reliable record for sub-fossil *Tilia platyphyllos* by Burchell and Erdtman (*Nature* **165,** 411; 1950).

On the North side of London, some unpublished analyses of the small valley mires of Epping Forest by P. Oxford have revealed high *Tilia* levels in basal samples. Now, in this issue of *Nature* (page 45), Girling and Grieg have published a pollen diagram from the Bagshot Sands of Hampstead Heath, and they also find high levels of lime pollen (up to 30% of arboreal pollen excluding *Alnus*). The exclusion of *Alnus* from the pollen sum has little effect upon these high *Tilia* levels below 100 cm in depth as *Alnus* abundance is low below this. On the basis of this and other data Girling and Grieg suggest that lime was a dominant tree in southern and eastern England. Locally this may well be the case, but the great variation found from one site to another does indicate that high *Tilia* representation is likely to reflect only local dominance. The isolated records of abundant lime pollen from sites further north and west, such as

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Shustoke, Warwickshire (Kelly & Osborne *Proc. Linn. Soc. Lond.* **176**, 37; 1965) and west Wales (Smith & Taylor *Trans. Inst. Br. Geogr.* **48**, 75; 1969) show that there may have been local pockets of lime dominance outside the south east of England. But until more data is available from southerly sites the extent of lime dominance will remain an area of speculation. Results such as those of Girling and Greig make it evident that many sandy areas in the south east which now bear heathland were once covered by forest at least locally rich in lime. ☐

Amyloidosis, P component and complement

from A. H. Gordon

AMYLOIDOSIS is the pathological deposition of glycoprotein-like material in body tissues. Various genetically different types of the disease have been identified, in which the major amyloid component and the sites of deposition differ. In primary amyloidosis (in which there is no obvious predisposing condition) immunoglobulins are apparently the main constituent of amyloid fibrils. In secondary amyloidosis, which is often a complication of chronic destructive infections such as tuberculosis and leprosy, the fibrillar AA protein deposits are immunologically similar to one particular plasma protein, SAA, (also found in normal sera) synthesised by B cells. Recent work on the analysis of amyloid tissue deposits has also revealed close amino acid sequence similarities between P component, present as a minor component in all forms of the disease and the recently discovered C1t, the fourth constituent of the first component of complement (C1). A more distant but definite sequence homology has also been demonstrated between P component and CRP (C-creative protein) (Levo *et al.* this issue of *Nature* page 56), a plasma protein which can interact with pneumococcal C-polysachharide, choline phosphate and various polycations to activate the complement cascade. Although a great deal is now known about amyloid components, the factors which cause amyloid deposition are still unclear.

In secondary amyloidosis for example, the concentrations of many plasma proteins, especially the so-called 'acute phase' proteins are increased, SAA among them. Acute phase proteins are those which are found in the serum in increased concentrations after any injury or shock. Amyloidosis can be induced in susceptible strains of mice by repeated injections of casein or lipopolysaccharide but McAdam and Sipe have shown that although injection of a single dose of bacterial lipopolysaccharide or casein (B lymphocyte mitogens) in mice leads to greatly increased concentrations of SAA in the plasma, this does not always lead to amyloidosis (*J. exp. Med.* **144**, 1121; 1976). They consider that in this case the difference between amyloid-prone and resistant mice is likely to be found in the lysosomes. According to this view the amyloid-prone mice are considered to suffer from a catabolic defect in protein degradation.

An interesting difference between SAA obtained from amyloid and non-amyloid human plasma has also been reported by Sipe *et al.* (*Br. J. exp. Path.* **57**, 582; 1976). This concerns the β-pleated sheet conformation which renders the protein of the fibrils insoluble. The difference relates to the dissociation in guanidine HCl or formic acid of SAA of molecular weight 160,000 to SAAL (molecular weight 12,500). In the same conditions SAA from amyloid plasma dissociated less, and reaggregated more readily, than SAA from normal plasma.

Because the increased concentration of SAA which occurs in these conditions will be accompanied by increased concentrations of a number of other plasma proteins, it is necessary to consider how far increased concentrations of other acute phase plasma proteins may predispose towards amyloidosis. In rabbits and humans the acute phase protein which increases most is CRP. As mentioned above, this protein shows partial amino acid homology with the P component of amyloid which is itself homologous with C1t. CRP, P component and C1t all complex strongly with Ca^{2+}. Furthermore, in inflammatory conditions the opportunity exists for CRP to complex with a number of cationic and/or anionic products of a cell breakdown or, indeed, with C-polysaccharide itself.

Such complexes act very similarly to antigen–antibody complexes in that they bind to C1 and initiate the consumption of complement. Perhaps the most surprising fact to emerge from the structural studies is the extensive homology between CRP, which itself is an activator of C1, and C1t, one of the subcomponents of C1. Since both CRP and C1t react with C1q they may in some manner modulate each others actions. This may affect the availability of P component and thus the likelihood of amyloid deposition.

These structural relationships, if seen from the evolutionary standpoint, take on a wider immunological significance. Also important in this regard is a further homology recently shown to exist by Osmand, Gewurz and Friedenson (*Proc. natn. Acad. Sci. U.S.A.* **74**, 1214; 1977) between CRP and the immunoglobulins. Comparison of the sequences of CRP and the C_H3 domain of human IgG has indicated a distant but significant homology. Using in addition data for C1t, the times since divergence of CRP and C1t have been calculated to be 2×10^8 yr, and more than 5×10^8 yr, for the earlier divergence of CRP from the immunoglobulins. The structural relationship now apparent between CRP and the immunoglobulins might perhaps have been expected in view of the similarity of function of antibody–antigen complexes and CRP when complexed with C-polysaccharide or another polyanion or polycation. These include the ability to stimulate phagocytosis and act both as agglutinin and opsonin. Particularly relevant to amyloidosis, however, is the activation of complement by both antigen–antibody complexes and CRP in complex form. Certainly long before divergence of CRP and C1t took place the parent molecule which may be presumed to have already had the power to bind Ca^{2+} would have been available as a constituent of amyloid.

Further evidence for the formation of amyloid from α_2 globulins of mouse plasma has been obtained by Kisilevsky *et al.* (*Am. J. Path.* **83**, 299; 1976). In this work with ^{125}I-labelled mouse plasma proteins, rates of synthesis and degradation of albumin α_1, α_2, β and γ globulins were measured for groups of mice receiving azocasein (AC) and amyloid-enhancing factor (AEF) obtained from the spleens of mice previously treated with AC. Amyloid was found only in the group which had received AC and AEF. However, both this group and that which had received AC only, showed significant increases in the turnover rate of α_2 globulin, $t^{1/2}$s 73% and 79% respectively, compared with the control group. This increased turnover of α_2 globulin by both groups can be reconciled with the appearance of amyloid in the AC+AEF group only, on the assumption that amyloid deposition occurs by removal of partially degraded material and that this process is without effect

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on the turnover rate. On the basis of these experiments Kisilevsky has attempted a generalisation as to the mechanism underlying amyloid deposition. In his view the precursors of amyloid are *l*-chains in myeloma-associated amyloid and α_2-globulin when the disease is secondary to an inflammatory state.

In such conditions an increased load of glycoproteins, which will be accounted for largely by the increased concentrations of SAA, will be taken up by the reticuloendothelial system (RES). If the RES is overloaded or functioning less than optimally, as for instance after treatment with BCG, degradation of α_2 globulins, and in particular SAA, will not go to completion. Thus, subunits will then accumulate and in due course will combine to form fibrils. However, for this to happen certain additional conditions are likely to be necessary. Since the partial breakdown of the SAA protein or other glycoproteins will occur in the lysosomes differences in respect to lysosomal enzymes are likely to be important. The existence of genetic differences in respect to amyloidosis has already been mentioned. Minor genetically-determined structural differences may perhaps be responsible for the enhanced tendency to associate as amyloid fibrils shown by the SAA obtained from amyloid plasma. ☐

Terminal redundancy in avian oncornavirus RNA

from T. J. R. Harris

THE avian oncornaviruses provide a good model for analysing the *modus operandi* of the Retroviridae. Viruses in this family make a DNA copy of their single stranded genome RNA using the RNA-dependent DNA polymerase (reverse transcriptase) packaged in the virus particle. This complementary DNA (cDNA) is then copied into a circular double-stranded DNA molecule which can integrate into the host cell DNA. Studies *in vitro* with reverse transcriptase are now beginning to unravel the possible molecular mechanisms behind this interesting sequence of events and to explain how the circular DNA molecules might be formed.

The primer required by reverse transcriptase for the initiation of synthesis is one of the host cell transfer RNA molecules associated with the genome RNA and making up the 70S complex in the virus. For both Rous sarcoma virus (RSV) and avian myeloblastosis virus (AMV), the tRNA specific for tryptophan is the one used. As cDNA synthesis proceeds in a 5′–3′ direction one might expect that the primer would be bound at the 3′ end of the RNA. Surprisingly, however, Taylor & Illmensee in a concise paper (*J. Virol.* **16,** 553; 1975) showed for RSV RNA that the primer was in fact bound near the 5′ end of the RNA—a finding since confirmed for AMV RNA (Stakus *et al. Virology* **71,** 162; 1976). Collett & Faras (*Proc. natn. Acad. Sci. U.S.A.* **73,** 1329; 1976) analysed the cDNA produced by the AMV enzyme and found that molecules containing sequences complementary to the 5′ end of the virus RNA also contained sequences complementary to the 3′ end. These observations led the authors to suggest that some of the sequence at the 5′ end of the virus RNA was repeated at the 3′ end providing both virus RNA and cDNA with the capacity to form circles. Direct evidence has now been obtained from sequencing studies to support this suggestion. Haseltine, Gilbert & Maxam (*Proc. natn. Acad. Sci. U.S.A.* **74,** 989; 1977) have sequenced the 101 nucleotides of tRNA^{Trp}-primed cDNA complementary to the 5′ end of the virus RNA using their rapid DNA sequencing technique, while Schwartz, Zamecnik & Weith (*Proc. natn. Acad. Sci. U.S.A.* **74,** 989; 1977) used oligo(dT)-primed reverse transcription followed by partial exonuclease digestion of the product to obtain a short sequence adjacent to the poly(A) at the 3′ end of the virus RNA. The first 21 nucleotides of RSV RNA after the capping group were found to be identical to the 21 before the poly(A). Haseltine *et al.* discuss in detail the implications of the terminal redundancy for circle formation and formation of provirus DNA and some evidence for the circular structures they envisage is accumulating from heteroduplex analysis of RNA/cDNA hybrids (see Junghans *et al. Proc. natn. Acad. Sci. U.S.A.* **74,** 477; 1977). Also, knowledge of the 5′ cDNA sequence enabled Haseltine *et al.* to construct plausible models of the possible ways that the 5′ ends of the two genome RNA molecules and their tRNA primers might be associated in the virus particle as the 70S complex.

The advent of the fast and accurate nucleic acid sequencing methods should enable more of the cDNA sequence to be determined (as well as that of the dsDNA and its precursor molecules) so that some of these speculations can be tested. One must add the proviso however that any mechanism of synthesis outlined from *in vitro* studies may not necessarily be the same as that actually occurring *in vivo*. Nevertheless, sequencing the parts of the RNA (by way of cDNA) coding for the structural and non-structural polypeptides will inevitably lead to a greater understanding of the replication of this important family of oncogenic viruses.

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Anoxic events in the Cretaceous ocean

from A. Hallam

THAT the Black Sea is stagnant in its deeper part is a widely known and comparatively well understood fact. Freshwater runoff from the surrounding land promotes salinity and hence density layering which is not destroyed by convective overturn because of restriction on interchange with Mediterranean waters by the sill of the Bosphorus. The surface waters contain free oxygen because of photosynthetic activity and exchange with the atmosphere, but at greater depth an anaerobic condition is produced as oxygen is consumed by organisms and replaced by hydrogen sulphide, the by-product of the metabolic activity of sulphate-reducing bacteria. In consequence the bottom sediments are rich in unoxidised bituminous matter derived both from terrestrial plants and phytoplankton, together with iron sulphide. They are also finely laminated because no benthonic organisms can survive in the anoxic environment and the sediment is not churned over by burrowers. Because of the name by which the Black Sea was known in the classical world, such sediments and environments are known as euxinic, and a Black Sea-type 'barred basin' model has been widely applied by geologists to account for the formation of finely laminated marine bituminous shales.

In marked contrast to the rather unusual Black Sea situation, the floor of the open ocean is well oxygenated,

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with powerful currents and water renewal operating even at great depth. It has therefore come as one of the great surprises of the Deep Sea Drilling Project that, at several horizons within the Cretaceous, bituminous sediments comparable with those of the Black Sea have been discovered beneath the Atlantic, Indian and Pacific oceans, with an organic carbon content as high as 28%. In the case of the earliest discoveries in the Atlantic, research workers initially proposed a Black Sea model, with stagnant basins in a much smaller ocean possessing only restricted connections with the larger ocean system. Now that it is known that such organic-rich deposits are much more widespread, and occur also on topographic rises in the Pacific, such an interpretation must be abandoned.

A number of recent papers is devoted partly or entirely to the problems posed by these deposits (Ryan & Cita *Mar. Geol.* **23,** 197; 1977: Schlanger & Jenkyns *Geol. Mijnb.* **55,** 179; 1976: Fischer & Arthur, *Soc. Econ. Min Paleont, Spec. Publ.* 25, in the press). On one major point all the authors are in agreement. The Cretaceous world ocean must have been in a very different state from that of today, with a considerably expanded oxygen minimum zone. The oxygen content in the modern ocean diminishes rapidly downwards in quite shallow water, but increases again in the depths. This pattern relates to strong circulation of cold and dense bottom waters from surface water at the poles and is therefore ultimately bound up with climate. In more equable periods in the past such as the Cretaceous, with no polar ice caps, oceanic circulation would have been much more sluggish, with a far lower rate of renewal of bottom waters.

Beyond this, the authors diverge in their treatment, though not necessarily in a mutually contradictory way. Ryan and Cita cite four principal horizons where extensive organic-rich sapropels have accumulated, in the latest Barremian, Aptian, mid-Cenomanian and latest Coniacian stages, and calculate that they contain an order of magnitude more carbon than is present in all the known reserves of coal and petroleum. They therefore form an enormous sink for carbon and also sulphur (because of the abstraction of iron sulphide). Ryan and Cita argue for significant increase, in consequence, in the amount of atmospheric oxygen. Schlanger and Jenkyns single out Aptian–Albian and Cenomanian–Turonian boundary beds as being especially important, and point to similar bituminous deposits occurring at these horizons also in quite shallow water deposits on the continents. They attribute these unusual and evidently extremely widespread occurrences to eustatic rises of sea level in a more equable world with a wider tropical belt than at present, with in consequence much higher organic productivity both on land and in the sea, and infer a pronounced oxygen minimum layer which reaches up to less than 300 m and down to 2,000–3,000 m below sea level.

For Fischer and Arthur, the Cretaceous bituminous deposits are an important component of an ambitious and imaginative hypothesis, which distinguishes a series of polytaxic and oligotaxic episodes extending back to the Jurassic with a 32 Myr cycle. Their polytaxic episodes are characterised by high organic diversity, higher and more uniform oceanic temperatures, eustatic rise of sea level, widespread marine anaerobism and undisturbed marine sedimentation, together with heavier carbon isotopes in organic matter. Oligotaxic episodes including the present day, exhibit, in contrast, lowered organic diversity, low sea level, sharper lateral and vertical temperature gradients in the oceans because of stronger circulation, more disturbed and aerobic sedimentation and lighter carbon isotopes. The fundamental controls are seen as climate and relative height of sea level.

To establish a 32 Myr cycle Fischer and Arthur seem to be guilty in some cases of pushing the evidence beyond reasonable limits. For example, they are obliged to stress the importance of the Albian bituminous horizon at the expense of the others, and choose to ignore the fact that in north-east England the stage is developed in red chalk facies, one of their markers for oligotaxic episodes. Furthermore, the Tertiary bituminous occurrences they cite are much less well established in the oceans than those of the Cretaceous. While there are indeed some striking correlations between diversity, sea level and isotope abundances in the Tertiary, the evidence presented for the Jurassic looks decidedly unconvincing. Nevertheless these reservations should not be held seriously to detract from a highly stimulating and thought-provoking work, which ought to be read by those interested in ocean history. □

New interest in carbon lamellar compounds

from A. R. Ubbelohde

A Conference on Carbon Lamellar Compounds was held at Napoule, near Cannes, on 23–27 May 1977. It was jointly organised by Professor A. Herold, Department of Applied Mineral Chemistry of the University of Nancy, and Professor F. Vogel, Moore School of Electrical Engineering of the University of Pennsylvania at Philadelphia. The Conference Proceedings will be published as a special issue of the *Journal of Materials Science and Engineering,* which will be obtainable separately. This article aims to stress some of the more striking information presented and discussed.

WAVES of innovative international research on graphites have passed through at least two previous maxima. The first wave was powered by interest in uses of graphite in nuclear energy generation. Gradually this has tapered to an asymptote, with the realisation that such uses have foreseeable limitations. A second wave was associated with the brilliant promise of carbon fibres for the aircraft industry. Following a spectacular setback, slow recovery of research and development on carbon fibres is under way. Though at present rather humdrum, and prompted by applications of carbon fibres to golf clubs and fishing rods, such background research seems likely to grow steadily.

A third wave of very active innovative research on graphites has formed internationally in recent years. It concerns the remarkable properties of their lamellar compounds. These are formed by intercalation of a great variety of molecules between the carbon hexagon layers in graphite, after these have been separated sufficiently for the intercalate species to enter and lie between them. Although the conference reported here dealt predominantly with graphite componds, other layer crystals such as MoS_2 show analogous behaviour. From a chemical standpoint, intercalates can broadly be grouped as electron donors, such as the alkali metals Li, (Na) K, Rb and Cs, and electron acceptors such as Br_2, ICl, inorganic halides, and a number of other types. There is also an important group of carbon lamellar

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compounds such as graphite bisulphate $C_{24}^{+}HSO_4^{-}2H_2SO_4$, formed by electrochemical (or the equivalent chemical) oxidation in the presence of the appropriate acid molecules.

Synthetic metals

One basic reason for the current widespread interest in lamellar compounds stems from the fact that in these the partner molecules form charge transfer bonds with the carbon hexagon networks, which act as giant aromatic molecules in this respect. Such charge transfer bonding was first postulated some 25 years ago (McDonnell *et al. J. chem. Soc.* 191; 1951). Many examples have now been studied. The striking growth of research on these compounds seems to be due to their interest and promise as synthetic metallic conductors. Intercalate molecules in carbon lamellar compounds also show distinctive chemical behaviour as catalysts, and in other ways. Means of making various precise physical and optical measurements have become more refined in recent years, so that much more precise information is beginning to be available to help interpret the nature and properties of the charge transfer bands formed. Nearly one-third of some 60 papers discussed during the meeting dealt with this aspect. Practically all the advances described were very recent. Surprising new facts and novel theoretical arguments kept members of the conference in a ferment throughout.

Inorganic chemistry

Experimental methods were described for introducing intercalate species not hitherto known to form lamellar compounds, for example by the use of oxidising agents such as $C_6F_6AsF_6$ (N. Bartlett, Lawrence Berkeley Laboratory, University of California, Berkeley) or catalysts such as $Co(AlCl_4)_2$ (E. Stumpp, Technical University at Klausthal-Zellerfeld). Production of C_8Li by interdiffusion of the powdered solid elements (Herold) is also a recent innovation in the use of Li as an electron donor intercalate. Formation of ternary lamellar compounds by intercalation of a second partner into binary lamellar compounds often occurs rather unexpectedly. Different instances described by several authors included the intercalation of molecular H_2, of tetrahydrofurane, of benzene, or of other molecules into lamellar compounds with alkali metals. Apart from any effects on the local charge transfer bonds, such ternary intercalation usually pushes apart even further the carbon hexagon layers from their initial separation of 3.35 Å in graphite. This 'layer dissociation' may have important consequences for electrical and other properties of carbon lamellar compounds.

Solid-state physics

Clarification of physical properties of various lamellar compounds of graphite was discussed. There have been elegant advances in band theory and important information has been obtained by precision measurements of various optical properties. For example, it now seems clear from infrared and Raman spectra that the fractional charge transfer when molecular Br_2 is intercalated is only a few tenths of one percent (G. Dresselhaus and M. S. Dresselhaus, Massachusetts Institute of Technology). Graphite/bromine was one of the first lamellar compounds for which a remarkable increase of metallic properties occurs on intercalation (McDonnell *et al. op. cit.*) and the rise in electrical conductivity begins steeply as the first few molecules of bromine enter the graphite. This recent optical work suggests (A. R. Ubbelohde, Imperial College, London) that metallic conduction in the halogen lamellar compounds resulting from charge transfer involves only relatively few additional charge carriers, but if so these must have exceptionally large mobilities.

Quite generally, under suitable conditions, synthetic metals formed by charge transfer in lamellar compounds of graphite seem to exhibit exceptionally low scattering of charge carriers travelling in the directions of the layer planes. This points to a two-dimensional mode of conduction which differs at least quantitatively and perhaps also qualitatively from that in the familiar three-dimensional metals (Ubbelohde). Such findings stress the importance of making many more measurements on the anisotropy of various physical properties parallel and perpendicular to the layer planes. Remarkably, very few *c*-axis measurements on lamellar compounds were presented to this conference, but there seems every hope that this gap will before long be filled.

A hundred years ago

THE application of new materials for paper stock which has occupied so much attention of late seems to have attracted some notice in the Philadelphia Exhibition last year. From Jamaica bamboo was perhaps the most important paper-making plant exhibited. Of the young bamboo stems, which are the best for the purpose, a very large supply, it is said, could be annually, by systematic cuttings or croppings, furnished from plants flourishing in the humid parts of the island.

from *Nature* **16**, 5 July, 198; 1877.

Ramsey fringes in optical spectroscopy

from Robin M. Hochstrasser

EXPERIMENTS have been reported recently in which the spectral resolution in optical spectroscopy of gases can be limited only by the time of flight difference between two light pulses. Limitations due to Doppler broadening, the homogeneous width of the resonant state, or the transform spectral width of the laser pulse, have been eliminated.

The Doppler broadening of a spectral line at ω_{ba} can be eliminated if the transition $a \rightarrow b$ is brought about by the simultaneous absorption of two photons, one from each of two counter-propagating beams having frequency $\omega_{ba}/2$. This type of non-linear spectroscopy requires high light power so that present day laser beams must be sharply focused in order for the signals to become readily detectable. With high peak power pulsed lasers such as a nitrogen-pumped dye laser some focusing of the beam is still necessary to observe these non-linear effects.

A severe limitation of pulsed dye lasers in high resolution spectroscopy is that typical pulses have a duration of 8 ns corresponding to a minimum frequency uncertainty of ~ 125 MHz for transitions occurring during the transit of such a pulse. For molecules travelling at 10^3 m s^{-1} across a laser beam focused to a diameter of 10^{-4} m the accuracy of the experiment is not limited by the transverse transit time which contributes only ~ 10 MHz to the spectral width. Transit time limitations on spectral accuracy have been known for a long time in beam resonance spectroscopy and in 1949 N. F. Ramsey (*Molecular Beams*, Ch V, Oxford University Press, 1956) suggested his method of separated oscillating fields in order to overcome this difficulty. In Ramsey's method the molecular beam passes through an RF field region of length l in time τ, and then travels a distance L before reaching a second identical RF field region. The number of transitions at a fixed RF frequency undergone in such a spectrometer is an oscillatory function of the time L/v, where v is the most probable velocity of molecules

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in the beam. In the intermediate region the separate components of the non-stationary state produced by the first RF region each evolve like stationary states for a time L/v so that the wavefunction is changed from

$$\psi(\tau) = \{a(\tau)\varphi_a + b(\tau)\varphi_b\}$$

into

$$\psi(\tau + L/v) = \{a(\tau)e^{-i\omega_a L/v}\varphi_a + b(\tau)e^{-i\omega_a L/v}\varphi_b\}.$$

The oscillations, or Ramsey fringes as they are now termed, arise because the probability of transitions in the second RF region is proportional to:

$$|A(\tau)\sin\{(\omega_{ba} - \omega)L/2v\} + B(\tau)\cos\{(\omega_{ba} - \omega)L/2v\}|^2,$$

which corresponds to an interference pattern. The fringes are separated by $V/2L$ whereas the spectra from only one RF region have widths of ~ $1/\tau$, so that the method of separated fields improves the sharpness of the spectral lines by a factor of ~ L/l.

In the paper by Salour and Cohen-Tannoudji (*Phys. Rev. Lett.* **38,** 757; 1977) an analogue of the Ramsey fringes was observed in the optical region. Whereas with the RF field it is easy to have two regions excited coherently during the whole experiment, it is rather difficult to accomplish this with optical fields. Instead, Salour and Cohen-Tannoudji generated two pulses in an optical delay line starting with a pulse obtained by amplifying a cw wave in a series of synchronously pulsed dye laser amplifiers. The two phase-locked pulses, separated by a distance L, were focused into a cell containing sodium vapour behind which was a mirror to reflect the beams back through the sample. The vapour was thereby subjected to a standing wave optical field of two pulses each of width τ separated by $T = L/c$. There is no Doppler shift for two-photon transitions in the standing wavefield. A photomultiplier detected the 90° fluorescence that occurs after two-photon absorption by Na atoms. The two-photon excitation spectrum of the $3^2 S \rightarrow 4^2 D$ transition, obtained by scanning the laser and detecting the fluorescence, showed not only the hyperfine structure, but each hyperfine component displayed Ramsey type fringes separated by as little as 30 MHz for $2T = 33$ ns.

Teets *et al.* (*Phys. Rev. Lett.* **38,** 760; 1977) also investigated the Doppler-free two-photon spectrum of sodium using a train of phase coherent pulses generated by multiple reflections of a dye laser pulse in a resonator containing the sample. The two photon signal was measured as a function of resonator tuning and a Fabry-Perot type spectrum was obtained with line-widths of about 12 MHz using a 2-m confocal resonator.

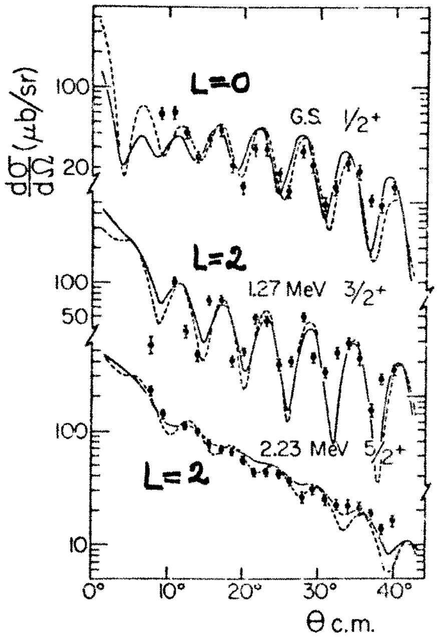
Although these experiments are expensive and not uncomplicated, especially in regard to the problem of maintaining a constant phase difference between the laser pulses, their success opens up important new possibilities for the study of molecular excited states at MHz resolution. ☐

Heavy ion transfer reactions

from P. E. Hodgson

THERE have been many studies of reactions between heavy ions in which one or more nucleons are transferred, and it is usually found that the cross section can be accounted for quite well by the distorted wave theory. In some cases it is found that the cross sections are more sensitive to particular aspects of nuclear structure than the corresponding reactions with light projectiles, and this makes them useful tools in nuclear spectroscopy.

An example of this is the spin-dependent effects in some transfer reactions. If a particle is transferred from a (l_2, j_2) state in the projectile to a (l_1, j_1) state in the residual nucleus the angular momentum selection rules allow several values of the transferred angular momentum L, and these depend on whether the angular momentum given to the residual nucleus is $j_1 = l_1 + \frac{1}{2}$ or $j_1 = l_1 - \frac{1}{2}$. In the particular case when $l_2 = 0$, then $L = l_1$ and it is possible to study the dependence of the cross section on j_1 for a given value of L.



Differential cross section for the reaction ^{28}Si(^{19}F, ^{16}O)^{31}P at 60 MeV to three states in ^{31}P compared with normalised distorted wave calculations including a spin-orbit potential in the incident and exit channels. The dashed curves show the effect of including inelastic excitations.

Such a study has recently been carried out by a group at the University of Minnesota for the ^{28}Si(^{19}F,^{16}O)^{31}P reaction at 60 MeV (*Phys. Rev. Lett.* **38,** 817; 1977). In this reaction three nucleons are transferred, but it is a good approximation to consider these as a triton-like cluster and thus retain the simplicity of a one-nucleon transfer reaction. This is likely to be quite a good approximation since 92% of the ^{19}F wavefunction can be expressed as a cluster of three particles in a relative 1s state moving with angular momentum $l_2 = 0$ with respect to the ^{16}O core. The experimental cross sections for the $L = 2$ transfers to the lowest $\frac{3}{2}^+$ and $\frac{5}{2}^+$ states in ^{31}P are shown in the figure; these differ markedly and show how the reaction can depend on j as well as on L. The cross section for the $L = 0$ reaction to the $\frac{1}{2}^+$ ground state is also shown.

The first distorted wave calculations, made with a finite range interaction and taking recoil into account, did not give a satisfactory fit to the data, and there was no appreciable improvement when inelastic excitation of the projectile was included by making a full coupled-channels calculation. The satisfactory fit shown in the figure was obtained when spin-orbit potentials were included in the distorting potentials in the incident and exit channels. Once again the fit was very little affected by the inclusion of inelastic excitations.

This work shows that the pronounced j-dependent effects in three-nucleon transfer reactions can be accounted for using the distorted wave theory provided spin-orbit potentials are included in the distorting potentials. It also provides striking confirmation of the validity of the cluster transfer approximation and shows that in this case inelastic excitations are relatively unimportant.

Further studies of similar reactions will be needed to confirm that the essential physics of the reaction has indeed been well understood, and if this is the case then the reaction, and others like it, should prove to be a powerful spectroscopic tool for determining the spins of the final states in the residual nuclei. ☐

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review article

Energy production by microbial photosynthesis

John R. Benemann*, Joseph C. Weissman, Ben L. Koopman & William J. Oswald

The large amounts of microalgae produced in waste treatment ponds during sewage purification are a potential source of methane and fertiliser. If techniques can be developed for the selective cultivation of filamentous or colonial microalgae, harvesting of microalgal biomass could be accomplished by low-cost straining or sedimentation methods.

INCREASING public and governmental awareness of the serious problems of energy and environment faced by most of the nations of the world is resulting in reappraisals of all potential energy sources. Evaluations of the various energy alternatives must consider, together with technical and economic factors, their environmental and social impact. Here we review the scientific and practical aspects of fuel and fertiliser production from microalgae and photosynthetic bacteria.

Bioconversion

Until less than a century ago, photosynthesis provided over 90% of mankind's fuels[1]. Even today, on a worldwide basis, the total calorific value of primary photosynthetic production (plant biomass) used by mankind is higher than that of the fossil energy consumed[2]. Only in the industrialised nations with temperate climates are fossil fuels more important than current photosynthetic biomass production in such a comparison. At present, over one-half of the people in the world depend on wood and dung for the majority of their fuels. The diminishing availability of these biofuels to the poorer half of the Earth's population is a more serious and immediate energy crisis than depletion or monopolistic control of fossil fuels.

Photosynthesis can provide a larger share of mankind's fuel needs through (1) shifts in relative usage (such as conversion of organic wastes to fuels or reduction in luxury consumption levels of animal protein) or (2) by increasing plant biomass production for direct utilisation or conversion to fuels. The potential of plant biomass production for the exclusive or primary purpose of fuel production is, in practice, limited by a number of constraints—the relatively low efficiency of photosynthesis in solar energy conversion; competition with agriculture or forestry for land, water and fertiliser; marginal economics of biomass production and conversion into usable energy; need for low-energy cultivation technology; and a lack of experience with such processes, and so on. Because most inputs in a bioconversion system would be constant per unit area, the net productivity (expressed as incident solar energy conversion efficiency) is a critical parameter since it must be sufficiently high to justify all costs.

Photosynthesis is limited by its dependence on visible light (about 45% of total solar radiation), reflectance losses (10–20%), a quantum efficiency of only 25%, and metabolic losses, giving a value of 5–6% for the theoretical maximum solar energy conversion efficiency[3-7]. In normal field conditions, photosynthesis rarely approaches the theoretical limit which applies only in light-limiting conditions. Sustained year-round, solar conversion efficiences exceeding 1% are unusual in agriculture. In addition, such high yields are often dependent on high fossil fuel subsidies or large human labour inputs, thus reducing the net efficiencies realised. Very high productivities (above 1% efficiency) with favourable net energy ratios are possible only with a few highly productive plants such as sugar cane[8]. The limitations to bioconversion suggest that practical applications will require utilisation either of the most efficient agricultural crops (for example sugar cane) or less efficient but more hardy plants capable of being cultivated in marginal areas with few inputs. The list of plants considered for biomass production is already large; they may be classified as either dry land or aquatic. The aquatic plants may, in turn, be classified as macro (water hyacinths, seaweeds, and so on) and micro (unicellular or small colonial algae and photosynthetic bacteria). We shall consider only the microscopic aquatic plants—microalgae and photosynthetic bacteria—and their potential in bioconversion to fuels, fertilisers, and petrochemical substitutes.

Microalgae in waste treatment

Large scale cultures of microscopic algae have been actively investigated for over twenty-five years[9]. Because of the high protein content of algae and the presumed large potential yields, algal research has emphasised protein production. However, practical applications of algal cultures for food or feed production are limited by high capital costs, moderate yields, poor product quality control, and lack of markets. At present, commercial production is limited to a one-ton-per-day pilot plant in Mexico producing *Spirulina* (a filamentous blue-green alga)[10] and several smaller operations in the Far East which produce *Chlorella*.

The development of large-scale algal ponds for domestic and industrial liquid waste treatment has been much more successful[11]. Although originally used only to hold wastes, ponds were observed to substantially reduce pollution indicators–biochemical oxygen demand, odour, and settleable solids. This turned out to be because pond algae photosynthetically generate enough oxygen to allow bacterial oxida-

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tion or organic wastes[12]. The released nutrients (carbon dioxide, ammonia, phosphate, etc) are in turn converted into algal biomass. Design equations have been developed to relate pond area requirements to sewage strength and flows, as well as local solar insolation and climatic conditions[13]. This engineering research has resulted in the widespread adoption of algal ponds (known as 'oxidation ponds') in many countries, including the United States, Australia, Israel, South Africa, and India.

Almost all presently used oxidation ponds are the 'facultative' type—one to three metres in depth and arranged in cells of up to 50 hectares, and mixed only by wind action and perhaps recirculation of effluent. The bottom of these ponds are thus anoxic, resulting in fermentation of the settled sludge and algae, while the surface is kept aerobic by the algae, preventing odours from developing (a prime consideration in sewage treatment). The liquid detention time (days required to displace one pond volume) is long, from weeks to months. This design is optimal for waste treatment but not for algal growth. If algal biomass production is to be maximised, the ponds must be shallow (30–50 cm), have short detention times (2–4 days in summer), and be mechanically mixed (to minimise algal settling and prevent thermal stratification). These types of ponds termed 'high-rate', are much more efficient solar energy converters and can be very effective in waste treatment, provided that the algae are removed before discharge of the pond effluent.

The high algal concentrations in pond effluents represent a load of organic matter and nutrients which are regarded by regulating agencies as environmentally undesirable. High-rate ponds produce well above $100\ \mathrm{mg\ l^{-1}}$ of algae (dry weight) and even facultative ponds normally have effluents of above $50\ \mathrm{mg^{-1}}$. These levels of suspended solids are higher than those allowed by present US waste treatment plant discharge regulations; thus, algae must now be removed from oxidation pond effluents. This step is, of course, also required for conversion of algal biomass into usable fuels.

Microalgal harvesting

Concentrating (at least 100-fold) a dilute suspension of microscopic algae is a considerable engineering problem[14,15]. Most oxidation pond algae are below $20\ \mu\mathrm{m}$ in diameter, too small for low-cost straining, filtration, or sedimentation. Only the more expensive centrifugation and chemical flocculation reliably remove algae from pond effluents. Centrifugation requires high capital investments in large centrifuges as well as power to run them. Chemical flocculation is somewhat more cost-effective and is being installed in a few large scale oxidation pond systems in the US, but the weight of chemicals (alum, lime or polyelectrolytes) required often surpasses that of the algae harvested. The flocculated algae are collected either by sedimentation or flotation and expensive extraction steps are then needed before the algae can be used for bioconversion. Furthermore, the chemical–algal sludge is difficult to dewater and dispose of. Thus, high capital and operational costs make chemical flocculation unsuitable for bioconversion systems. Less expensive methods of removing algae from effluents are to settle them in the pond by chemical flocculation, or by prolonged (2–3-week) isolation of ponds from further sewage flows. These methods, although economical, are not suitable for algal biomass production since the algae settle on the pond bottom as a thin layer of sludge which slowly decomposes.

Water reservoirs often exhibit blooms of algae which impart foul tastes and odours to the water and which are removed by large scale 'micro-strainers'. Almost all such algae occur as long filaments or large colonies, which accounts for the effectiveness of microstrainers—rotating straining devices (Fig. 1)—in the purification of reservoir waters. Oxidation pond algae, on the other hand, tend to occur as unicellular or small colonial types, generally under $20\ \mu\mathrm{m}$ in any one dimension, which are too small to be effectively strained. Only the sixth and seventh most common algae in oxidation ponds—the filamentous blue-green alga *Oscillatoria* and the colonial spined green alga *Micractinium*[16], can easily be harvested by microstrainers. If such types of algae were normally prevalent in high-rate oxidation ponds, microalgal harvesting would no longer be a limiting economic factor in algal biomass production from liquid wastes.

Algal population dynamics

Control of algal species in ponds (whether for biomass harvesting, aquaculture, or protein production), must be based on an understanding of the specific factors affecting algal population dynamics. The following are a few generalisations about the importance of various factors. When high strength wastes (for example, food processing wastes) are serially diluted, inoculated, and incubated in the light, different biotypes will appear in the various dilutions. The most concentrated wastes will contain only anaerobic fermentative bacteria. Photosynthetic bacteria appear in the first dilution followed by a graduation of flagellated euglenoids, blue-green algae, green algae, and finally diatoms and nitrogen-fixing blue-green algae. Such successions can also be seen in outdoor ponds going from heavily loaded anaerobic ponds to the final 'polishing' or 'tertiary' ponds. Often, only one or two algal species comprise over 95% of the algal biomass in sewage ponds that are not allowed to fully stabilise (through fast dilution and heavy organic loading). A greater number of algal species is found in the more lightly loaded tertiary ponds, indicating greater stabilisation of the wastes and algal populations. The filamentous blue-green alga *Oscillatoria* is usually found at higher temperatures (being the predominant algae reported from Indian oxidation ponds) and in ponds receiving wastes low in nitrogen (such as cannery wastes). The many environmental, biological, operational and even historical factors which affect algal population dynamics in ponds interact in many ways, complicating the task of understanding algal population dynamics. For example, is is known how seeding of ponds with algae from operating oxidation ponds will affect the establishment and subsequent history of algae in the ponds. In outdoor systems, it is not possible to achieve a steady state due to constantly changing environmental conditions. Temperature, for example, is an important factor in algal successions[17]. The greatest problem is the lack of detailed ecological or limnological data from operating oxidation ponds. The low species diversities and rapid succession of the predominant algal species in ponds indicate a versatile system which can be affected by many environmental conditions.

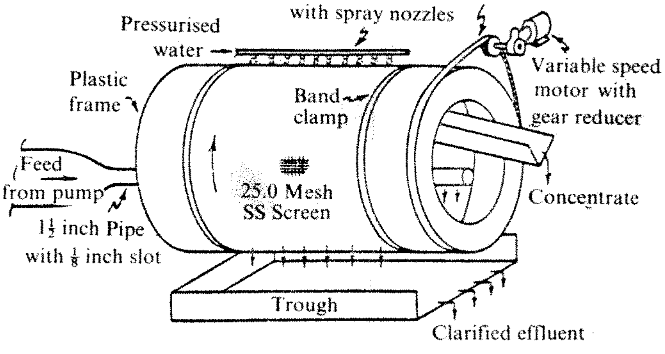

Fig. 1 Small-scale microstrainer

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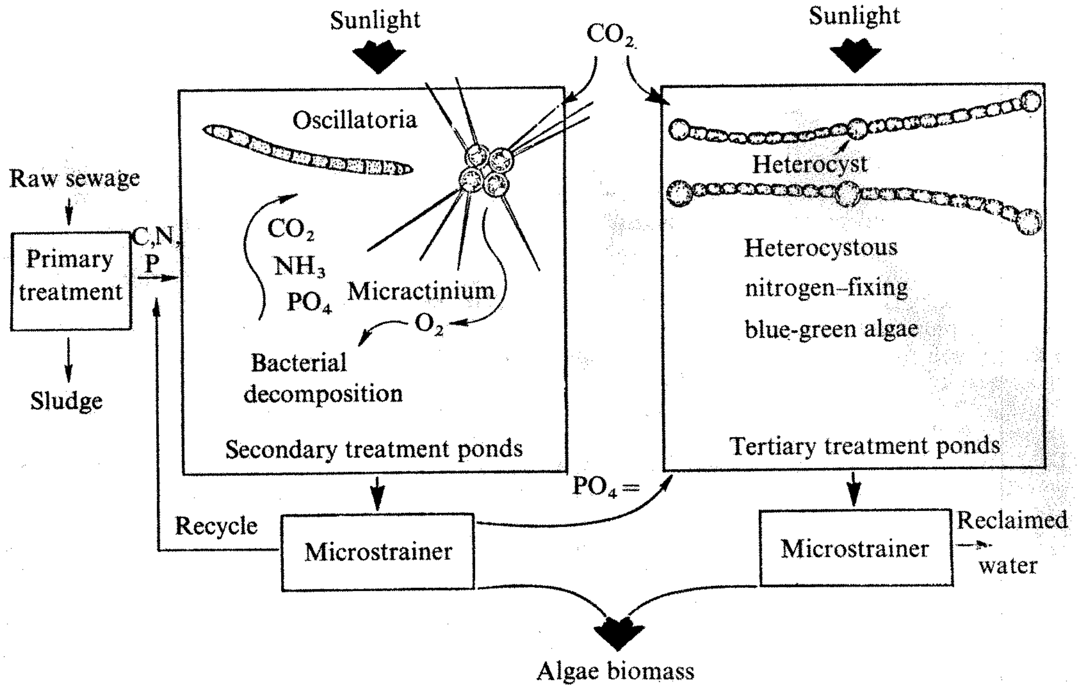

Fig. 2 Two-stage algal ponds for complete liquid waste treatment.

Algal species control

How, then, can algal populations be controlled so as to maintain selected algal species (or algal types) for prolonged periods? Sunlight and temperature cannot be significantly controlled, although they are critical to the succession of algal species. Pond loadings, depth, and mixing can be controlled, however; their particular effects on algal species are not yet known and would be influenced by additional environmental conditions. Thus, the selective species control methods must be adjustable in the face of changing conditions. Two such methods are presently being studied at our laboratory—size-selective recycle, and nutrient limitations.

The following is an example of size-selective recycle. In a pond operated at a five-day detention time, the predominant alga *Chlorella* is accompanied by a small percentage of *Micractinium*. *Chlorella* is the preferred food of many zooplankton such as *Daphnia*, whereas *Micractinium* is seldom grazed because its spines, which protrude in all directions (Fig. 2), obstruct ingestion by rotifers and similar zooplankton. The zooplankton cannot flourish because their population growth cannot keep up with the short detention time. If a relatively coarse screen were used to remove the *Daphnia* from the pond effluent and recycle it into the pond, a population explosion of *Daphnia* would be predicted to cause a diminished *Chlorella* population and thus lead to predominance of the undigestable *Micractinium*. Such biological control of algal species has yet to be demonstrated in ponds.

A more direct method of size-selective recycle has been used in outdoor ponds to achieve algal species control.[18] It involved recycling to the pond a certain fraction of the algae harvested by a microstrainer. If a pond is heavily inoculated with harvestable species and all the algae strained from the pond are re-introduced into the pond, these algae, because they are not washed out of the pond, will have an advantage over faster-growing but non-harvestable algae. Eventually, most of the algae in the pond will be of the desired, harvestable type and the amount recycled to the ponds can be decreased. To prevent non-harvestable algae from returning, the fraction of harvested algae that must be continuously recycled is proportional to the ratio of the relative growth rates. In practice, this recycling fraction must be larger since harvesting is neither completely selective nor totally effective. Thus, the growth rate of the desirable harvestable filamentous or colonial algal species must not be too far below that of the unwanted single-cell algae. The faster dilution rates or higher algal concentrations required by cell recycling also set limits to the allowable degree of recycling. It is important to note that such recycling is a size-specific, not a species-specific control method. Other strains of harvestable algae which spontaneously appear in the ponds will be recycled and become dominant if they grow faster than the inoculated strain. Inoculations and recycling might be alternated so as to allow true species control. The principles of selective biomass recycling are also applicable to any other harvesting method (such as settling) and to other large scale microbial processes including activated sludge, fermentations, and single-cell protein production. Indeed, in the activated sludge process, the practice of returning settled bacterial biomass to the reactor results in selection for settleable bacterial flocs.

The other method now under investigation for establishing harvestable species in ponds is based on nutritional limitation. The major nutrients of algae are water, sunlight, carbon, nitrogen, and phosphorus. The degree to which any one of these nutrients is limited will affect algal populations. To maximise biomass production, sunlight should be the only limiting nutrient, since productivity is proportional to solar energy conversion efficiency. However, the final yield of algae will be determined by the limiting nutrient in the wastes, which for domestic sewage is carbon. Treatment of sewage and other liquid wastes cannot be considered complete until nutrients causing eutrophication in receiving waters (N or P) are removed from sewage treatment plant effluents, and that means adding an additional source of carbon to algal ponds. Carbon dioxide produced by a power plant or any other source could easily be brought to ponds for this purpose and would allow growth of the harvestable algae (maintained through selective recycle) up to the nitrogen algal growth potential of the sewage. An additional crop of algae could be obtained from the residual phosphate in the effluents of these ponds through further carbonation. In such a final algal production and phosphate stripping pond, only nitrogen-fixing algae could grow (Fig. 2). Nitrogen-fixing algae are blue-green and all planktonic forms are filamentous. Thus, they are easily harvested and, since growth is selective, no recycling would be required.

Wherever phosphate detergents are used, nitrogen-fixing blue-green algal biomass can be produced in large quantities (exceeding the production of the initial ponds), thereby increasing the potential N fertiliser output from the ponds to a level above that present in the wastes.

Algal bioconversion

In the multi-pond system of waste treatment and algal biomass production proposed above and indicated in Fig. 2, two ponds in series (in practice several more would be required) are used to grow successive crops of different algal types harvestable by microstrainers or similar inexpensive methods. In such ponds, energy and plant nutrients are recovered from wastes and augmented by the processes of carbon dioxide enrichment and nitrogen fixation. The most direct and practical method for conversion of the algal biomass would be methane fermentation. Direct burning is not feasible since it would require dewatering of algae, a process requiring more energy than the algae could yield.

More than one-half of the heat of combustion of the algae (10,000 BTUs per lb algae) can be converted into methane gas with digester loadings, temperatures, and detention times similar to those of sewage sludge[20,21]. Many problems remain, including the possible resistance to degradation by part of the algal biomass, ammonia build-up in fermenters, and dewatering properties of the residual sludge. Of particular concern is the cost of conventional sludge digesters; covered anaerobic ponds might prove to be the most cost-effective large scale digestion systems. The production costs of methane would be part of the overall sewage treatment costs, making it a valuable by-product of algae-based waste treatment systems. The residues from algal digestion would contain virtually all the nitrogen and phosphorus of the algal biomass. These could be disposed of as fertiliser on agricultural land. In principle, all necessary agricultural fertiliser could be produced in this way—one acre of algal ponds providing the fertiliser required by 10 to 50 acres of modern agriculture.

The total amount of methane that can be obtained from the algal biomass produced during sewage treatment is limited to only a few percent of local natural gas needs. It has been demonstrated in laboratory studies that algae can be cultivated on the residues from algal methane fermentations.[22] Therefore, by recycling the fermentation residues to the algal growth ponds algal biomass production can be expanded to the limits of available water and land. However, in such cases, no waste treatment or fertiliser production credits would help underwrite the economics of methane production. In principle, capital investments (into pond construction) and operational costs (including energy inputs) could be low enough to allow large scale algal biomass production and conversion systems. The feasibility of such large scale 'nutrient integrated' systems will depend on the solution of many technical and economic problems.

Algal productivity

So far we have emphasised the problem of algal harvesting, species control, and bioconversion because they must be solved before large scale microalgal biomass production can become feasible. However, it is the harvestable yields of algal biomass (expressed as gm per m^2 per day or MT per hectare per year) that determines the potential of algal systems for energy and fuel production. There is no strong theoretical basis for expecting higher yields with algae than with higher plants. Vertical shade adaptation of leaves in a crop plant is inherently a more efficient arrangement than the uniform pigmentation of algae in a mixed water column. The reflectance of sunlight from the water surface is another disadvantage of algal ponds. However, algal ponds have several compensating features, primarily the capacity for continuously supplying all nutrients so that only light is limiting. Thus continuous culture makes possible a quick, graded response to environmental conditions and complete light absorption. The hydraulic nature of a pond system allows for simple and inexpensive operations. However, continuous cultivation also means harvesting of a dilute suspension of the microalgae. This is inherently more expensive than the periodic harvesting possible with macroalgae or land plants.

On the basis of even such a qualitative analysis, it may be concluded that rates of algal biomass production would not be necessarily higher than those observed in intensive agriculture, algal production, however, being potentially less demanding on inputs and efforts. It is not possible at present to make comparisons—many reported peak agricultural productivities are suspect on a number of accounts (methodology, border effects, and so on), and algal productivity is normally based on extrapolations from short term, small scale experiments. A figure of 50 MT per ha per yr (12 gm m^{-2} d^{-1}) of dry-weight, ash-free algal production has been presented as a reasonable figure of potential productivities[19]. This would represent a total solar energy conversion efficiency of about 2% at 30° latitude. It might be possible to perfect algal pond systems with higher conversion efficiencies. This will require careful pond operations and genetic selection of the algal strains to be cultivated. For example, the addition of carbon dioxide to ponds could be used (in addition to the reasons listed above) to maintain an adequate *p*H in the ponds (it may go above 10 in the afternoons, resulting in a sharp decline of photosynthesis). This would not only extend the daily growing period, but also reduce photorespiration and the potential for photooxidative death[23]. Another factor which could increase photosynthetic efficiencies and which may account for some of the very high yields reported is the phenomenon of photoheterotrophy. Many algae assimilate preformed organic compounds, particularly under low light intensities (as may be expected in the bottom of ponds). Since little light energy is required in the photoheterotrophic production of biomass, calculated efficiencies are high because the energy content of the liquid wastes are ignored. Controlled photoheterotrophic growth would increase the performance (load rates, organic waste destruction, and biomass production) of the primary ponds receiving settled sewage. Photosynthetic bacteria are, of course, photoheterotrophic by nature and are potentially useful in waste treatment systems. Another suggestion for improving photosynthetic efficiencies would be through decreasing the accessory pigment concentration of the algae to decrease self-shading and photoinhibition.

An important aspect to be considered is the reduction in net photosynthetic efficiencies resulting from energy inputs into pond operations such as dilution, mixing, fertilisation, harvesting and concentrating, species control, and so on. If the large scale production of algae for energy production is to be feasible, these inputs must be minimal. A slight net energy gain has been calculated from waste-grown algae using available production methods[24]. In principle, net algal productivity should not be limited by the energy requirements of cultivation which are low; mixing at slow speeds (about 5 cm s^{-1}) is sufficient for thermal destratification and requires little energy[25,26]. Harvesting by microstrainers is likewise not energy intensive. Water usage in pond systems is mainly through evaporation; the ability of microalgae to grow in brackish or saline waters would make it possible to use unreclaimable water exhaustively through use of a terminal evaporative pond system. Fertilisation obviously would be too expensive if mineral or synthetic fertilisers are used; nutrient recycling, whether from wastes or internally, is required. Nitrogen fixation must make up extra nitrogen requirements at a probable reduction in photosynthetic efficiencies estimated at 10%. Including energy inputs and conversion losses, a net production of 200 M BTUs per acre per year of methane seems to be a feasible goal[19,27].

Future developments

The use of microbial photosynthesis for energy production is not limited to methane and fertilisers. Biophotolysis—the decomposition of water to yield hydrogen gas—has been demonstrated with algae in the laboratory[28,29]. Heterocystous blue-green algae can be used to produce hydrogen and oxygen simultaneously in a system of horizontal glass tubes[30]. Other algae might be capable of alternating daytime oxygen with night-time hydrogen production. Photosynthetic bacteria can produce hydrogen from organic substrates[31], presenting a potential alternative to methane fermentation[32]. The production of specialised chemicals is possible. The large scale culture, of the salt-loving alga, *Dunaliella,* consisting of 80% glycerol, has been proposed (M. Aaron personal communication). Algal species containing high concentrations of fats and lipids may be used for liquid fuel production. Petrochemical feedstocks of various types may theoretically be derived from algae. However, such technologies are probably going to take longer to perfect.

Microalgal bioconversion will have a beneficial impact on environment and society as long as it does not conflict with food production or develop into gigantic centralised conversion systems. The prospects of very large scale sites (hundreds or thousands of square miles) converted to pond systems is not imminent; technological development will certainly at first favour smaller locally integrated waste treatment-algal biconversion-nutrient recycling systems. These would be of particular value where adequate insolation is combined with a need for sanitary sewage disposal and fertiliser supplies. Such pond systems may expand, as practicable, to dispose of salt-laden agricultural drainage water by evaporation and to provide a significant fraction of local fuel needs. Energy production from microalgae might have little immediate overall impact on energy consumption of advanced industrial countries, except where favourable local situations exist. Algal bioconversion is specifically important to underdeveloped countries since such technology would require little or no investments in scarce raw materials or complex 'hardware'. The application of sophisticated cultivation technology should be within the reach of even the poorest countries. The environmental and social impacts of algal pond systems or other bioconversion technology are expected to be minor compared with present or alternative energy sources. In the future, the developing technology of bioconversion can present mankind an alternative to depleting fossil fuels and dangerous reliance on nuclear energy.

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articles

Lateral mobility of human erythrocyte integral membrane proteins

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Fluorescein isothiocyanate-labelled integral membrane proteins are mobile in the membranes of human erythrocytes that have been fused (and haemolysed) by Sendai virus or polyethylene glycol. Minimum diffusion coefficients are of the order of 10^{-11} *cm*^{2} *s*$^{-1}$ *at 37 °C. This mobility is reduced several-fold at room temperature, not detected at 0 °C, and is significantly greater in fresh than in aged blood. Mobility was assessed by observing the spread of fluorescence on labelled cells which had been fused with unlabelled cells; neither intramembrane particle aggregation nor spectrin release occurred during this process.*

FACTORS which regulate the lateral mobility of cell surface components have been described for a number of cells[1,2], but the complexity of these cells makes it difficult to study the relevant interactions at the biochemical level. The erythrocyte membrane is more readily accessible to direct biochemical investigation but it is generally thought that lateral movements of proteins in this membrane either do not occur or are extremely limited[3,4]. Antigenic components, visualised using direct and indirect immunofluorescent staining[4,5] and fluorescein-conjugated lectins[6], have not been observed to patch or cap in membranes of adult erythrocytes from various species. Similarly, the intramembrane particles exposed by freeze-fracturing are not readily aggregated in the intact erythrocyte[7,8]. These particles represent proteins intercalated into the lipid bilayer[9,10],

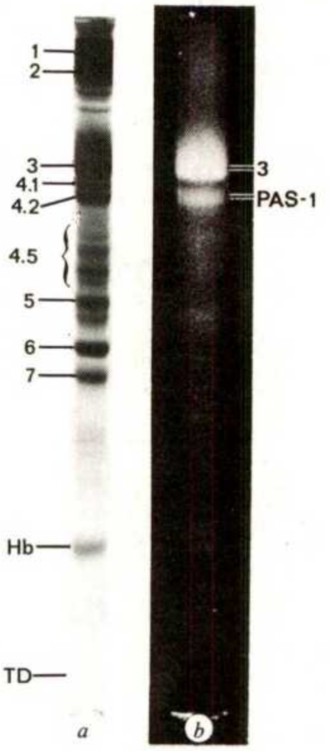

Fig. 1 A 7.5% SDS–acrylamide gel of fluorescein isothiocyanate-labelled erythrocyte membranes. *a*, Proteins stained with Coomassie brilliant blue. *b*, The same gel before Coomassie blue staining, showing fluorescent labelling. Intact erythrocytes were labelled by a modification of the method of Peters *et al.*[15]. Outdated human blood stored in acid–citrate–dextrose (about 3–6 weeks) was washed twice in 310 mOsm phosphate-buffered saline, *p*H 7.6 (PBS), removing the buffy coat after each wash, and then twice in 145 mM NaCl–10 mM $NaHCO_3$, *p*H 9.5. The packed cells were resuspended in an equal volume of a 5 mg ml^{-1} suspension of an FITC–cellite adsorbate (Sigma) in the same buffer, and agitated overnight at 4 °C. The cells were resuspended in 50 mM glycine–95 mM NaCl–10 mM $NaHCO_3$, *p*H 9.5, to stop the reaction, and layered over a $\rho = 1.13$ metrizamide shelf (32% diluted to 23.8% in the NaCl–bicarbonate buffer). After centrifugation the cells were collected from the interface, the celite having sedimented to the bottom of the tube. The cells were washed once again in the glycine–bicarbonate buffer, then twice in PBS. Ghosts were made according to Dodge *et al.*[25], omitting the saline and isotonic phosphate buffer steps. Gels were run according to Fairbanks *et al.*[26]. The fluorescent gels were photographed while still in the tubes, using a long wavelength ultraviolet lamp for excitation, and a yellow barrier filter with Tri-X film. The negative was scanned with a Joyce–Loebel densitometer, and comparison of the areas under the peaks revealed that greater than 70% of the total label was present in the Band 3/PAS-1 region (> 50% in band 3). No fluorescent material ran in front of the tracker dye (TD), and less than 10% of the label migrated immediately behind the tracker dye, where both free FITC and some lipids migrate in this gel system. The fluorescent band just below Band 3 on these 7.5% acrylamide gels could be readily identified as PAS-1 by its altered mobility on 5% gels[27].

and have been attributed to the principal erythrocyte integral membrane proteins, Band 3, and the major sialoglycoprotein[10–14]. Peters *et al.*[15], labelled a heterogeneous group of components in the human red cell membrane (both proteins and lipids were labelled) with fluorescein isothiocyanate, and measured the rate of fluorescence recovery after bleaching one half of the erythrocyte ghost. They observed essentially no recovery of the bleached half in the conditions of the experiment, implying that the labelled components, if diffusing at all were diffusing only very slowly in the plane of the membrane.

But, prostaglandin-induced changes in erythrocyte membrane lipid fluidity detected using electron spin resonance techniques[16,17], paralleled by changes in membrane circular dichroism spectra[18] and in the rheological properties of the blood cells[16,18], indicate that both lipid fluidity and protein–protein associations may play a dynamic part in the cell's response to its environment. Two recent reports indicate that surface antigens of intact human red cells can indeed undergo redistribution in the plane of the membrane. Clustering of fluorescein-labelled anti-blood group A antibodies has been correlated with a change in erythrocyte morphology from a biconcave disk to a crenated sphere[20], and a temperature dependent redistribution of concanavalin A (con A) bound to the cell surface, induced by anti-con A antibodies, was visualised directly on the erythrocyte surface by freeze-etching[21]. Here we report results which demonstrate a time and temperature dependent mobility of labelled proteins in the plane of the adult human erythrocyte membrane. The basic strategy of these experiments was suggested by Frye and Edidin's initial demonstration of antigen intermixing in mouse–human heterokaryons[22].

Membrane labelling and cell fusion

Membrane proteins which have been operationally defined as integral[23,24] were labelled with fluorescein isothiocyanate (FITC) (Fig. 1). After fusion (and haemolysis) of labelled and unlabelled cells with Sendai virus or polyethylene glycol (Fig. 2), the proportion of fused cells which was uniformly fluorescent increased with time except at 0 °C (Fig. 3); this is due to spreading of label over the surface of the initially bright-dark cells. Although only the end points were scored, all degrees of fluorescence distributions from bright on one half and dim on the other, to a barely distinguishable difference between the two halves, were observed.

Redistribution of label was not due to movement of fluorescently labelled haemoglobin or other cytoplasmic components because when fused lysed cells were washed in 20 mOsm phosphate buffer, *p*H 7.6, resuspended in 310 mOsm phosphate-buffered saline, *p*H 7.6, and then incubated at 30 °C (fluorescent haemoglobin was only about 1% of the total labelled protein in such ghosts) label redistribution proceeded as in unwashed cells. (This procedure was not used routinely because hypotonic lysis and subsequent resuspension in isotonic buffer resulted in shrinkage and crumpling of the membranes, making it difficult to positively identify the fused cells.) Furthermore, treatments which

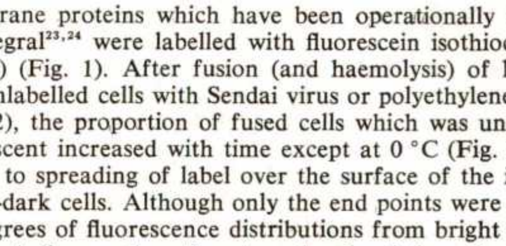

Fig. 2 An FITC-labelled cell fused with an unlabelled cell, incubated for 2 h at 22 °C. Haemolysis accompanied fusion. *a*, Fluorescence photograph. *b*, Phase contrast photograph of the same cell. ×1,280. Cells were fused by Sendai virus as follows. A 50/50 mixture of a 5% (v/v) suspension of labelled and unlabelled cells in PBS was added to an equal volume of PBS containing between 5,000 and 10,000 HA per ml of egg-grown Sendai virus. This was shaken at 150 r.p.m. at 0 °C for 15–20 min to agglutinate the cells, and then at 37 °C for 10–12 min. The fusion step was terminated either by addition of an equal volume of ice-cold 310-mosM PBS while vortexing rigorously to break up agglutinates, or by washing the cells once in about 10–15 volumes cold PBS (SS-34, 10,000 r.p.m., 30 min, 4 °C) with subsequent resuspension in an appropriate volume of PBS. As a control in some experiments, cells were fused with a 50% polyethylene glycol (molecular weight 6,000) solution by a modification of the procedure of Davidson and Gerald[28] for fusion of cultured cells in suspension. Fused cells which survived this process behaved as cells fused by Sendai virus.

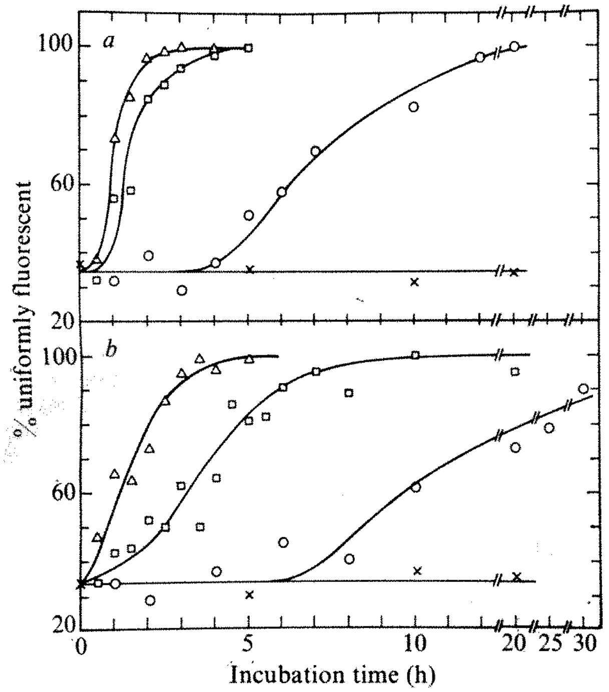

Fig. 3 Redistribution of label on FITC-labelled cells fused with unlabelled cells. Uniformly fluorescent fused cells expressed as % of the total number of fused cells which are at least partially fluorescent. *a*, Fresh blood. *b*, Aged blood stored 3–6 weeks in acid–citrate–dextrose at 4 °C. △, 37 °C; □, 30 °C; ○, 23 °C; ×, 0 °C. Note the breaks in the time scale at long incubation times. Cells were fused by Sendai virus as described in Fig. 2. After fusion, the cell suspensions were divided into four portions, incubated at the indicated temperatures, samples taken at various times and kept on ice until counted. To prevent bacterial growth, 0.5 mM NaN_3 was routinely included during the incubations; curves for redistribution of label were identical in the presence and absence of azide. Fused fluorescent cells were scored as uniformly fluorescent, that is, no difference in intensity was distinguishable on one half of the fused cell relative to the other half, or as not uniformly fluorescent, that is, some difference was visible. A total of 100 cells from each sample were scored for the determination of each point; points from one representative experiment are plotted. Note that at 0 time one third of all fluorescent fused cells are uniformly fluorescent; these result from the fusion of two initially fluorescent cells with one another. This is the expected outcome of a fusion process in which it is equally likely for any cell to fuse with any other and in which only fluorescent fused cells are counted.

remove the so-called 'peripheral' proteins[23,24], such as low ionic strength extraction, high salt washes, *p*-chloromercuribenzoic acid, or 10% acetic acid treatments[24,29], failed to elute any fluorescence from ghosts or fused cells. Spreading of label is also not due to movement of dye molecules which have simply adsorbed on to or partitioned into the membrane, since less than 10% of the total fluorescent label migrates in the position of free fluorescein isothiocyanate on sodium dodecylsulphate (SDS)–acrylamide gels (Fig. 1). In addition, FITC–celite pre-incubated in 50 mM glycine–95 mM NaCl–10 mM $NaHCO_3$, *p*H 9.5, did not label the cells, and all cells were washed in this buffer after labelling (Fig. 1).

Fusion was always accompanied by haemolysis. But, neither proteolysis nor extensive intramembrane particle aggregation could be detected in Sendai virus-treated erythrocytes. SDS–acrylamide gels of haemolysed and fused cells incubated at 37 °C until label had become uniformly distributed were identical to gels of ghosts from untreated cells (Fig. 1) in terms of fluorescent labelling and staining with Coomassie blue. SDS–gel electrophoresis is sufficiently sensitive to detect proteolysis of ghost membrane proteins by microgram per millilitre quantities of trypsin[30,31]. Moreover, proteolysis leads to vesiculation of the ghosts[30] and clustering of the intramembrane particles[14], which were not observed after treatment by Sendai virus. Samples of the supernatant and the pellet of cells fused and incubated in the same buffer without resuspension were electrophoresed in parallel. Supernatants to which was added an amount of spectrin (bands 1 and 2) and actin (band 5) corresponding to less than 10% being released from the cells into the lysate provided a standard for comparison, and demonstrated that the treatments used in our experiments did not result in detectable spectrin (or actin) release from the membrane. It was important to rule this out, since the ability to induce redistribution of the intramembrane particles has been correlated with previous treatments which dissociate the peripheral membrane proteins spectrin (and actin) from the cytoplasmic surface of the membrane[8]. In addition, curves plotted for label redistribution in polyethylene glycol-fused cells (not shown) correspond closely to those for Sendai virus-fused cells (Fig. 3).

Membrane protein mobility

The abrupt increase in uniformly fluorescent haemolysed and fused cells after a short lag time is particularly striking at physiological temperatures. Decreasing the temperature to 22 °C dramatically slows the rate of label redistribution, and further reduction of temperature to 0 °C prevents it completely. It is evident from the curves obtained that some fused lysed cells become uniformly fluorescent sooner than others. This heterogeneity is most clearly visible at lower temperatures where the rates of label movement are slower. Evaluation of variations in the rate of label movement at physiological temperatures is difficult, due to the errors involved in determining rates by scoring a population of fused lysed cells for each time point. Direct determination of rates on individual fused cells by measuring the increase in fluorescence intensity on the initially dark half of the fused cell would clarify this question.

Approximate diffusion coefficients for minimum rates of label redistribution have been calculated (Table 1). Using fresh blood, Peters *et al.*[15] set an upper limit on the diffusion coefficient for fluorescein isothiocyanate-labelled components in the erythrocyte ghost membrane at 3×10^{-12} cm^2 s^{-1} at 22 °C, which compares favourably with our value of 6×10^{-12} cm^2 s^{-1}. The diffusion coefficients which we calculate at 37 °C are about an order of magnitude greater, however, and approach those determined for plasma membrane proteins in other cell systems, which fall between about 10^{-11} and 10^{-10} cm^2 s^{-1} (refs 33–38). The possibility that the fusion and lysis process itself affects the lateral

Table 1 Minimum diffusion constants determined for FITC-labelled integral membrane proteins in the erythrocyte

Temperature (° C)	Fresh blood (cm^2 s^{-1})	Aged blood (cm^2 s^{-1})
37	4×10^{-11}	2×10^{-11}
30	3×10^{-11}	1×10^{-11}
23	6×10^{-12}	2×10^{-12}
0	No movement of label observed	

Approximate diffusion coefficients for the integral proteins of the slowest cells to show complete label redistribution were determined from the data in Fig. 3. The formula developed by Huang[32] for diffusion from one half of a spherical shell into the other was used, assuming first that the time course of label redistribution on these cells can be approximated by such an exponential decay process, and second that the fused cell doublet was a sphere with a surface area twice that of a single cell (verified by measurement on photographs of fused cells). We further assumed that the time for label equilibration on the slowest cells was the time at which at least 90% of the initially bright–dark fused cells had become uniformly flourescent, and that such fused cells would appear uniformly fluorescent to the human eye when twice the relaxation time for label equilibration in a fused doublet had elapsed (compare Fig. 2 in ref. 32).

mobility of the labelled proteins cannot be ignored. It is significant, however, that the diffusion coefficient inferred for membrane antigens at 37 °C in mouse–human heterokaryons produced by fusion with Sendai virus[22,23,32] is within the range of values we have found in fused red cells. A pronounced dependence on relatively small variations in absolute temperature seems to be characteristic of the lateral mobility of membrane proteins[22,39], in contrast to the far more rapid diffusion of membrane lipids, which is relatively unaffected by corresponding variations in temperature[33–38]. Factors such as membrane viscosity, phase transitions and separations of the membrane lipids, boundary lipids associated with a particular protein, and dynamic interactions with other proteins, within or beneath the bilayer[2], almost certainly regulate the lateral mobility of these membrane proteins.

Table 2 Effect of an ATP regenerating system on the rate of redistribution of label

Time of incubation (min)	% Fluorescent fused cells uniformly fluorescent			
	Fresh blood		Aged blood	
	+ATP	−ATP	+ATP	−ATP
0	36	36	32	32
30	40	36	—	—
60	75	52	44	32
90	92	68	64	44
120	100	98	84	52

Freshly drawn blood was washed thoroughly and aged to reduce intracellular ATP levels by incubation at 37 °C for 16 h in 135 mM NaCl, 20 mM $NaHCO_3$, 3 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, and 200 μg ml^{-1} benzylpenicillin, *p*H 7.6 (ref. 41), and then labelled as for Fig. 1, simultaneously with freshly drawn blood from the same donor. Cells were fused with Sendai virus as for Fig. 2, the suspensions were divided into two portions and washed once in 310 mOsm PBS, *p*H 7.6, as described in Fig. 2, and then resuspended in either PBS (−ATP), or PBS plus 1 mM ATP, 2 mM $MgCl_2$, 5 mM phosphoenolpyruvate, 20 μg ml^{-1} myokinase, 50 μg ml^{-1} pyruvate kinase, *p*H 7.6, (+ATP), and incubated at 37 °C as described in Fig. 3. The omission of either phosphoenolpyruvate or ATP from the regenerating system gave results which were not significantly different from those in PBS alone.

The marked differences in rates of label equilibration for fused lysed cells made from fresh blood as compared with aged, ATP-depleted[40] blood suggested that intracellular ATP levels might affect the lateral mobility of the membrane proteins. Incubation of the fused lysed cells in the presence of an ATP regenerating system increased the rates of label redistribution significantly (Table 2). Since cells from both aged and fresh blood are invariably haemolysed during fusion and are thus equally depleted of metabolites, and since the presence of an ATP regenerating system does not decrease the length of time for label equilibration to equivalent values for both fresh and aged cells, factors in addition to energy availability probably control the lateral mobility of these membrane proteins. Cell shape, deformability, and divalent cation content change dramatically during cell ageing and ATP depletion[40,41], and may have an important role in these complex phenomena.

Discussion

Observations of parameters influencing the lateral distribution of the intramembrane particles exposed by freeze-fracture have led to the notion that lateral movements of the integral membrane proteins are restricted by a spectrin–actin meshwork beneath the erythrocyte membrane, either by a direct linkage mechanism or by trapping between the interstices of the meshwork[8,24,42]. As pointed out before[8], however, aggregatability of the intramembrane particles must be distinguished from direct measurements of protein mobility. The importance of distinguishing between particle aggregation and mobility becomes evident if it is borne in mind that treatments which induce particle aggregation perturb the membrane. Such perturbations may alter dynamic interactions between proteins so as to favour associations that restrict mobility.

Moreover, although the intramembrane particles seem to consist, at least in part, of Band 3 and the principal sialoglycoprotein [10–14], they may not coincide with the fluorescently labelled proteins whose mobilities we observe at a light microscopic level. Our data do not exclude the possibility that some of the proteins in the erythrocyte membrane are less free to diffuse than others, since the rates of label movement which we observe represent an average, both over a population of cells and a population of integral membrane proteins. But, if all the label present in the Band 3 region (at least 50%, see Fig. 1) were to be immobilised with respect to the other labelled components, we would expect that at the end point, the population of fluorescent fused cells would consist of approximately 33% uniformly and brightly fluorescent cells and 66% in which three-quarters of the label is on one half of the fused cell and one-quarter on the other half. This is not the case. A report that the rotational diffusion of Band 3 proteins (labelled with eosin isothiocyanate) is not affected by removal of most of the spectrin from erythrocyte ghosts[43] is additional evidence that protein mobility and particle aggregation are controlled by different factors. Specific and dynamic interactions between spectrin[44], or other peripheral membrane proteins, and the integral membrane proteins may regulate the lateral mobilities and associations among proteins in the plane of the membrane. This could provide a potential mechanism for the dynamic interaction between extracellular events and internal metabolic processses in the erythrocyte.

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Plate linkage mechanism to account for oroclinal deformation in the Western Cordillera of North America

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Plate tectonic theory is used to predict the motion of small plates which remain coupled within discrete regions to adjacent large plates. Taking the Western Cordillera of North America as an example, it is shown that the three oroclines situated between Vancouver and Northern California are very largely removed when that portion of the orogenic belt south of the Klamath mountains is rotated back in time with the Pacific plate while retaining the northern Cordillera as a rigid portion of the American plate. The rotation at the Pacific–America pole is found to be 14.4° which suggests that the deformation began during the Oligocene.

PLATE boundaries are generally well defined, both seismically and topographically. When traced out around the globe they are seen, in most cases, to divide the surface into a number of discrete plates each decoupled from its neighbours. There are, however, regions where the boundaries cannot easily be delineated, for example, in parts of the Mediterranean or the southerly continuation of the East African Rift. There may be various reasons for the uncertainty including, in some instances, a lack of seismicity. There is also the possibility, however, that some of these regions are more complicated than is implied by a simple plate tectonic interpretation. Wilson[2] envisaged plate boundaries to be of three types which are currently referred to as spreading ridges, subduction zones and transform faults. Thus a ridge may change or be 'transformed' into a fault or subduction zone at what he called a transform. To these three we may add a fourth type of boundary where the plates become attached to one another. Clearly this situation can only exist very briefly because of the rapid build-up of stresses induced by the relative motion between the plates. Such a situation must have existed in Baja California when this portion of the North American plate was transferred to the Pacific[3]. Here the outcome was relatively straightforward and with new transform faults and intervening ridge segments developing to the east of Baja to accommodate the strain. In other similar instances an alternative process may sometimes have occurred whereby the two original plates remain joined by a sliver of lithosphere which is hinged to each. Consider for example, the plate shown in Fig. 1*a* which is being split apart by rifting. If instead of a single fracture, there develop two major fracture zones, neither of which is able to completely truncate the plate. Motion of the northern half in a north easterly direction pulls the sliver away from both the northern and southern plates. Assuming the sliver to remain rigid except in the vicinity of the hinging points, then finite motion cannot occur until a third hinge pole labelled γ in Fig. 1*b* becomes established somewhere between the other two. For any defined finite rotation between the two major plates, there must exist a unique solution to the orientation of the small 'linked' plates.

Over 20 years ago S. W. Carey noticed that, while some orogenic belts are dominantly linear in plan view, others show sharp inflections[4]. The question which he posed then and which remains today is whether such bends were part of the orogenic belt at the time it was forming or whether they formed later hence representing what he called 'an impressed strain'. He assumed the latter interpretation to be correct and defined the term orocline for such bends. We may see how the concept of hinging small plates to large ones can explain the formation of oroclines by considering a hypothetical linear orogenic belt labelled ABCD in Fig. 2*a*.

The northern portion A is kept fixed and the southern portion D is allowed to move with resepect to A. The pole AD is placed at various points on the stereogram and in each case the rotation angle of D to A is 20°. To accommodate this rotation without rupturing the orogen, two link plates are permitted to form, namely B and C. The kinks which subsequently appear, due to the rotations, are shown in Fig. 2, *b*–*f*. The poles AB and AD are fixed with respect to the stereogram while BC and CD are instantaneous. The latter two poles may, however, be located since the distances between the hinge poles are known. Poles AC and BD are instantaneous with respect to the stereogram and to all four plates. They may be located by graphical means using the

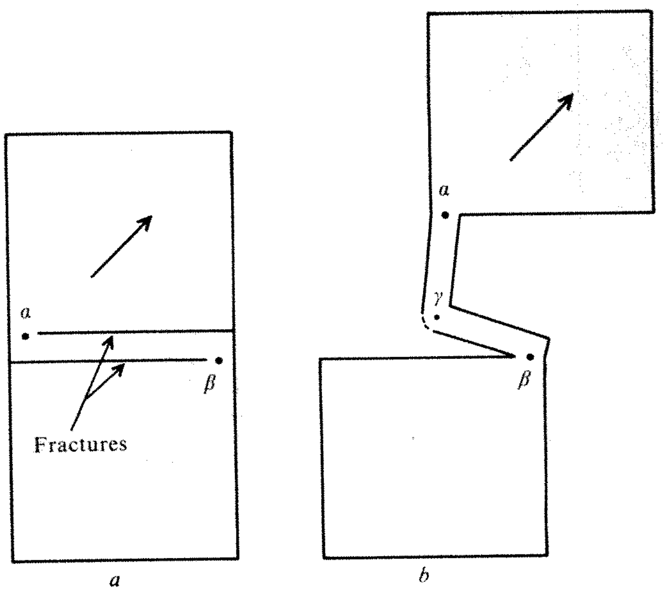


Fig. 1 One of several ways in which linked plates may form. *a*, The northern portion of the plate begins to pull away towards the north east causing fractures. These fractures do not sever the plate but instead leave the fractured central portion joined at α and β. *b*, With finite motion the distance α to β decreases causing the interconnecting plate to buckle at a third point γ.

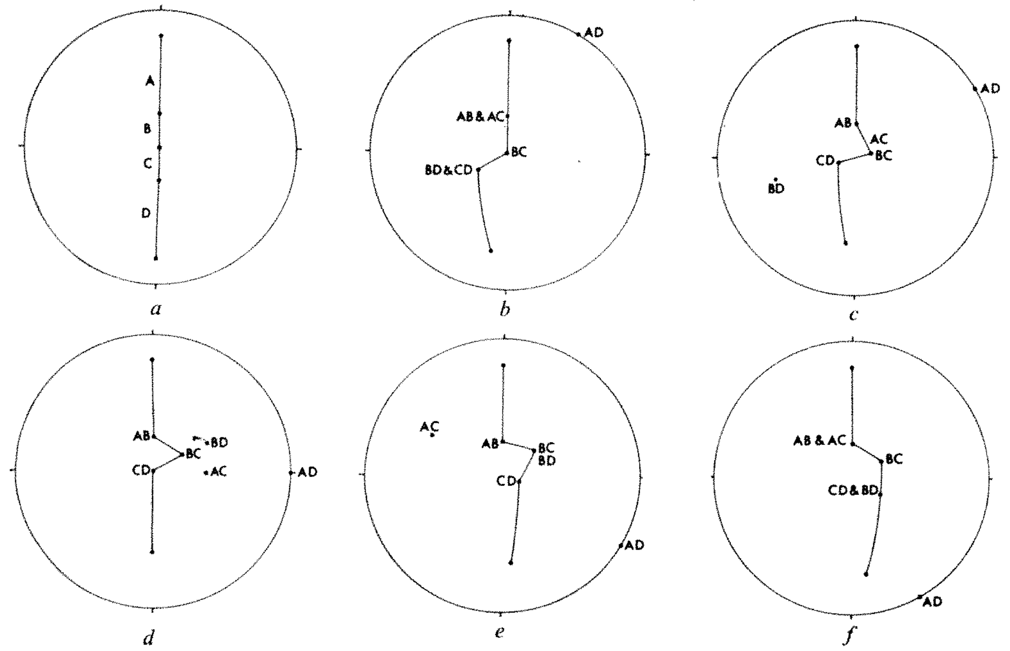


Fig. 2 Diagram illustrating how different oroclinal configurations may develop depending on where the pole of relative motion for the driving plates is located. *a*, Initial condition with linear orogenic belt ABCD. *b–f*, Oroclines generated by placing the pole AD at five different points on the great circle which forms the equator of the Lambert equal area projection.

property that the three poles for any triple plate problem always lie on a great circle. Thus considering, for example, the pole BD in Fig. 2; this occurs at the intersection of the two great circles passing through first CD and BC and also that through AB and AD. Not only are the locations of poles BC, CD, AC and BD instantaneous, but for a given constant rate of pole AD, rates at all the other poles vary continuously.

Carey realised that oroclines do not usually occur in isolation and grouped them together in pairs[5]. In doing so, he recognised left lateral and right lateral shear as the cause and defined the structures as being sinistral and dextral coupled oroclines respectively. In applying the plate tectonic interpretation outlined above, we see that it is impossible for rigid plates to rotate about two hinge poles and it follows that either the lithosphere is not behaving rigidly in these conditions or else the oroclines should occur in sets of three. Where three oroclines are present, the terms dextral and sinistral are inappropriate, however, it is apparent that the deformation shown in Fig. 2 could have proceeded in such a way that the hinge pole BC moved out of line towards the west rather than to the east.

The Cordillera along the western margin of North America show several sharp inflections in the trend of both the orogenic belt and the outcrop pattern of the associated granitic intrusions. These bends have been interpreted as being due to dextral shear by several authors including Carey (refs 5–7). The evidence in support of these bends being secondary is from palaeomagnetism and from various branches of geology. Palaeomagnetic results from the Siletz River volcanics in Oregon[8] and the Columbia Plateau basalts[9] all show a clockwise rotation subsequent to their formation. The geological evidence includes the trend of the structural grain of the rocks which is seen to follow the outcrops of the orogenic belt and is hence deflected through large angles at the oroclines. Other evidence comes from the turbidites of the Tyee formation in Western Oregon which in their present configuration, are seen to have flowed northwards into an oceanic embayment in the continental margin rather than to the west into the Pacific[10].

An examination of the geological map of North America shows the orogenic belt to be dominantly linear between latitudes 60°N and 52°N. Near Vancouver Island however, there is a sharp bend which deflects the belt along with the granite plutons through approximately 30° and this is taken to be the third orocline which, along with those identified by Carey[5], is required in order that these bends may have developed by linkage of rigid plates. Further south the orogen is seen to bend in Idaho from a NW to SE trend between Vancouver and Idaho to almost NE to SW betwen Idaho and the Klamath Mountains. South of the Klamath Mountains the belt apparently runs in a SSE direction parallel to the Sierra Nevada; however the late Tertiary volcanic rocks in the Cascade Mountains and the sediments in the Great Valley may conceal the fact that relative motion has occurred between these two regions during or after the period of oroclinal deformation.

If the bends are accepted as oroclines then the driving mechanism can only have come from the relative motion between the Pacific and American plates. It is envisaged that a small portion of the latter became rigidly attached to the Pacific plate in mid Tertiary times when that segment of the Farallon plate between the Pioneer and Murray fracture zones was completely consumed in the subduction zone[3]. The process was probably essentially similar to the incorporation of Baja California into the Pacific plate but in this instance the relative motion did not enable this piece of the Cordillera to become completely sheared off from America. Instead, the compression was transmitted all the way up the orogenic belt as far as, or even past, Vancouver and it was forced to buckle at the three weakest points. Once these kinks or hinge poles became established, then it should have been possible for the deformation to proceed in such a fashion that the segments of the Cordillera in between remained rigid. The edges of these segments should then have followed the rules of plate tectonics from which we may predict the nature and amount of compression and/or extension between them and the American plate.

In accordance with Carey's practice of giving names to the oroclines, I shall refer to the hinge poles in order from north to south as Vancouver, Idaho and Klamath respectively. Unlike other poles of relative motion which are merely the geometrical consequences of the motion of rigid plates, hinge poles have considerable geological significance since they represent the sites of profound tectonic activity. The linked plates in between these poles will be referred to as the Washington plate north of the Idaho hinge pole and the Oregon plate between the Idaho and Klamath poles.

To restore the orogen to its original shape, the required parameters are the coordinates of the three poles and the coordinates and rotation angle of the pole of finite relative

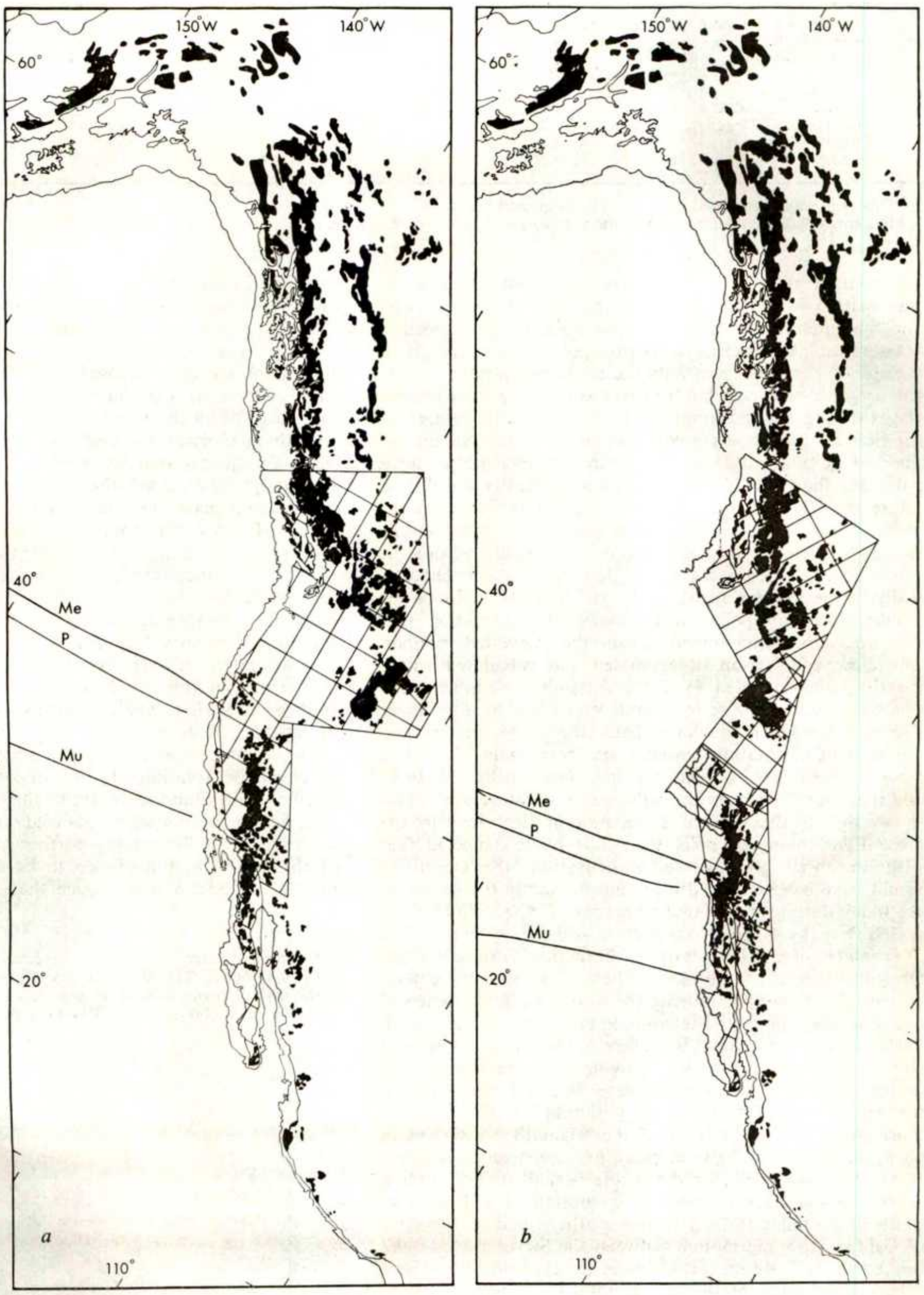


Fig. 3 The western margin of North America showing: *a*, the present configuration of the proposed linked plates and *b*, the Mid Tertiary (approximately 30 Myr ago) orientation of these plates as predicted by the model described in the text. Areas shaded in black are Mesozoic granitic intrusions. Fracture zones on the Pacific plate are Me, Mendocino; P, Pioneer and Mu, Murray.

motion between the Pacific and American plates for the period in question. The locations of the poles between the linked plates have been estimated from the tectonic map of North America[11] and are listed in Table 1. The youngest magnetic anomalies between the Pioneer and Murray fracture zones vary from 10 in the north to 6 in the south[3]. This suggests an age of between 32 and 26 Myr BP as the time of onset of oroclinal deformation while the opening of the Gulf of California 4 Myr ago[12] may be taken as marking the end of that deformation. The pole and angle for the finite motion during this period cannot be determined directly and indirect methods using circuits involving perhaps five or more plates lack precision[13]. For these reasons, I shall use the best available data for the instantaneous relative motion[14]. Even more uncertain than the location of the pole is the rotation angle for the period. Molnar and Atwater have shown, using plate circuits, that the instantaneous rate was significantly slower in mid Tertiary times than at present and their reconstruction for 29 Myr ago suggests that a rotation of about 14° has occurred between then and the beginning of the opening of the Gulf of California. Instead of using this angle, we may proceed to determine the rotation required to line up the three hinge poles on a great circle, this being the maximum possible angle as further rotation would require the orogenic belt to have been longer before the oroclinal

Table 1 Estimated rotations taken from maps compared with predicted values using the proposed plate tectonic model

Plate pair	Pole		Rotation angle (°)	
	Lat.	Long.	Estimated	Predicted
America–Washington	51.0°N	127.0°W	30	31.4
Washington–Oregon	45.5°N	115.0°W	110	114.0
Oregon–Pacific	42.0°N	123.5°W	80	70.8
Pacific–America	52.0°N	76.0°W*	14†	14.4

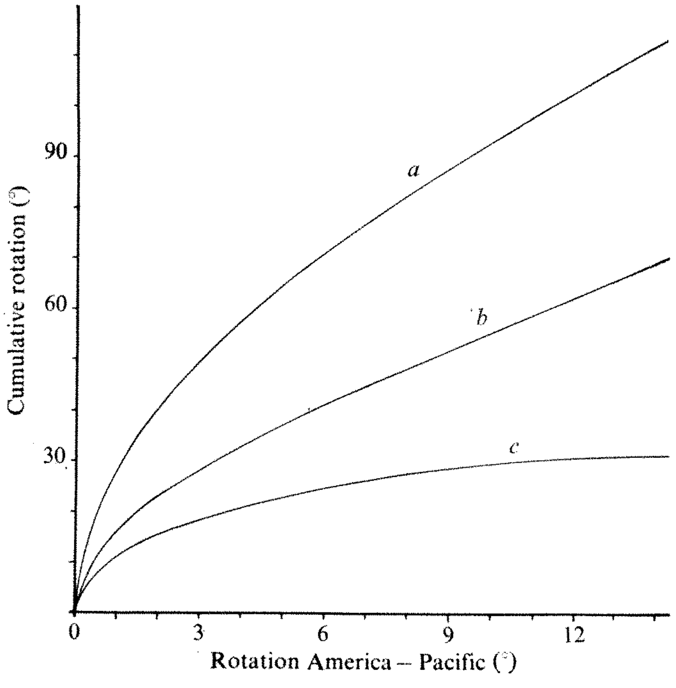
*This pole was determined by Chase and is quoted in ref. 14.
†This angle has been determined by the writer using Chase's pole and the 29 Myr b.p. reconstruction in ref. 13.

deformation than it is at present. A computer program was written to determine the locations of the hinge poles and the amount of rotation at these poles for any specified rotation at the Pacific–American pole. Using this it is possible to determine not only the angle required to line up the orogenic segments but also to examine the intermediate stages during the deformation. It was found that 14.4° of rotation about Chase's pole[14] was required to line up the three hinge poles; the rotations which are incurred at these poles are shown in Table 1. It is geometrically possible to rotate the Klamath pole about any arbitrary point on the globe to line up the three hinge poles on a great circle. However, such rotation would not, in general, restore the whole orogenic belt to a great circle configuration. Theoretically, there exists a great circle which is the locus of the Pacific–American pole which satisfies the condition that the orogen be straightened out in the suggested manner. The close comparison of estimated and calculated values for the rotation listed in Table 1 tends to confirm that Chase's pole lies on, or is at least very close to, this locus. The distance along the locus from the orogen determines the amount of rotation required and here again, there is a close resemblance between the calculated value of 14.4° and that estimated from the published reconstruction[13]. This shows that if the pole of finite motion lies close to the present instantaneous pole, then it is to be expected that with an initial great circle configuration, the Cordillera would have been distorted into almost exactly the shape we see today during the allotted time span of about 25 Myr.

Before making the reconstruction which is shown in Fig. 3, estimates of the boundaries of both the Washington and Oregon plates had to be made. These must, to some extent, be tentative, however, moving them to a limited extent will not seriously modify the tectonic implications of the model. Perhaps the most critical boundary is the one between that part of California which was moving with the Pacific, and its contact with the American plate. It is not obvious from the geology where this boundary should be placed but it must have been to the east of the Klamath Mountains in northern California. It is unlikely to have been to the east of the Sierra Nevada for the whole period of deformation as this would have opened up a substantial gulf further south in a similar fashion to the southern end of the Gulf of California. Some motion between the Sierra Nevada and the Basin and Range province is here assumed to have occurred, however, as this can account for the late Tertiary dilation and consequent faulting in this region. By analogy with the present San Andreas transform fault, it is likely that the boundary in question was also a fault and this may now be largely concealed by the sediments of the Great Valley. The most likely location, however, seems to be in the metamorphic belt of palaeozoic and mesozoic strata on the north-western flanks of the Sierra Nevada batholith. Here faults with a suspected strike slip component[15] may be traced over 300 km from the Cascade Mountains in the north to the eastern margin of the Great Valley where presumably they continue under the late Tertiary sediments.

We may now examine the nature of the relative motion between the linked plates and the American plate in greater detail by dividing up the total rotation at the Pacific–American pole into 24 increments of 0.6°. In doing so, each increment represents approximately 1 Myr. The cumulative rotations at the hinge poles are shown in Fig. 4 while in Fig. 5, lines joining these poles at every third increment are superimposed on a simplified geological map of the area. It can be seen that the rates of rotation decreased at all three poles with time from which it follows that the geological processes associated with these rotations must also have varied accordingly. If we take, for example, the compression along the Rocky Mountains between the Washington plate and the American plate, it is found that about 90% of the total compression was accomplished during the first half of the period. From the migration path of the instantaneous pole between the Oregon and American plates (see Fig. 5) it is predicted that initially there would have been compression along the entire boundary between the two. The early compressive phase at the southern end may at present be represented on the American plate by the belt of metamorphic rocks to the west of the Sierra Nevada referred to earlier. The outcrops show a bend of about 25° at latitude 37°N which if due to oroclinal deformation could be interpreted as being caused by the initial high rate of clockwise rotation of the Oregon plate relative to the Pacific. With time the nature of the motion on the Oregon–American plate boundary changed with extension beginning in the south and migrating northwards. During the last half of the period it is predicted to be dominantly extensional throughout and it is envisaged that at this time the Sierra

Fig. 4 Cumulative rotations at the three hinge poles as predicted by the model. The slope at any point on these curves gives the instantaneous rate in relation to the American–Pacific rate. Curve *a*, Idaho pole; *b*, Klamath pole; *c*, Vancouver pole.



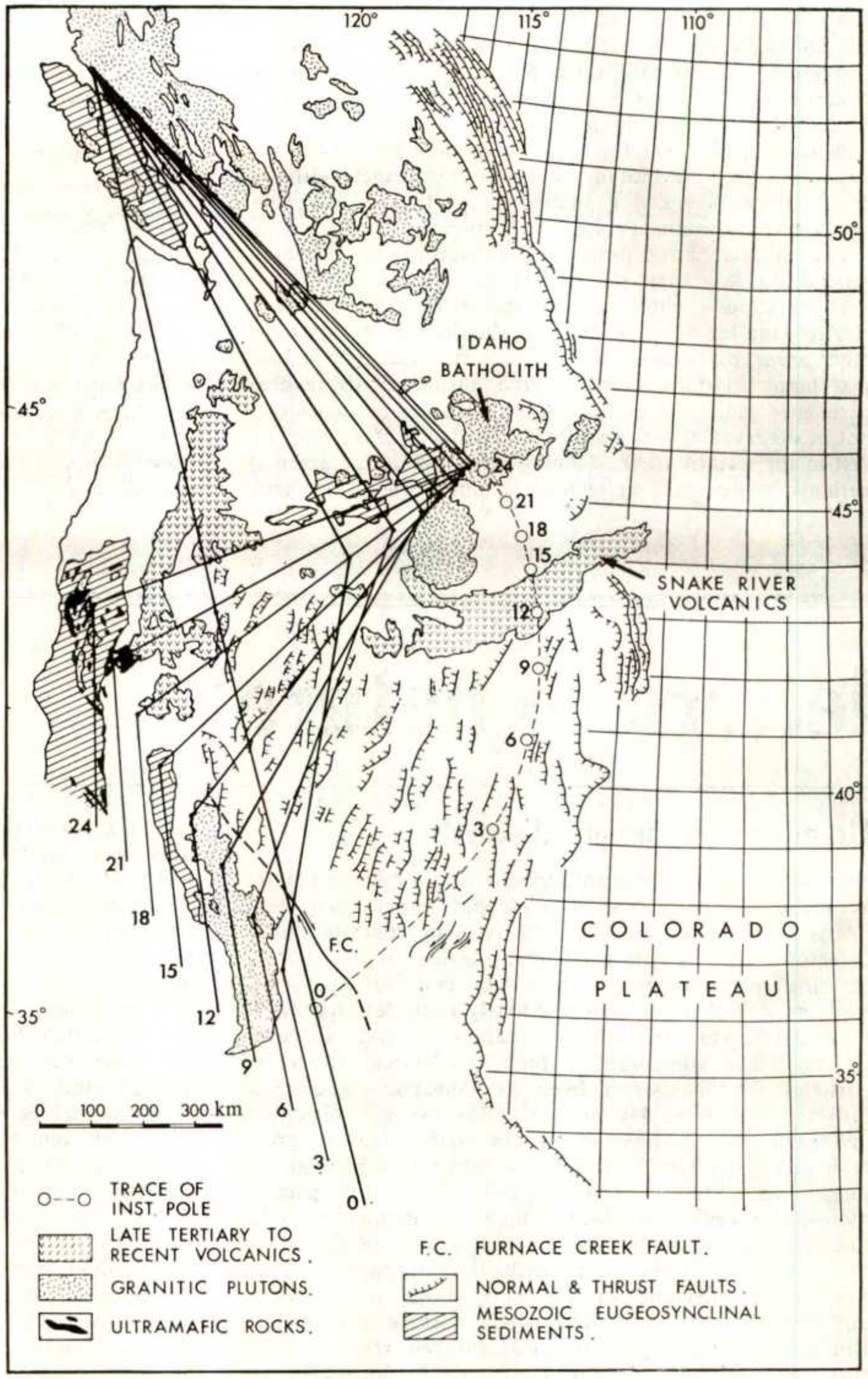


Fig. 5 Simplified geological map of the region showing the predicted motions of the linked plates and hinge poles in relation to the American plate. For clarity only a third of the 24 increments which were used to construct Fig. 4 are shown and each therefore represents a rotation of 1.8° between the American and Pacific plates. The trace of the instantaneous pole across the Basin and Range province is that for the motion between the Oregon and American plates.

Nevada region moved with the Pacific plate. In doing so, it would have moved north-westwards relative to the Basin and Range and it is likely that most of the motion was taken up on the Furnace Creek fault. Tension was generated in two distinct areas at this time namely, the Great Basin (north of latitude 36°N) and the Southern Basin and Range (between latitudes 36° and 28°N). In the former case, the relative motion was controlled by the Oregon–American pole and there is some indication that the normal faulting in this region has a radial distribution about a point in central Idaho (see Fig. 5). The implied degree of extension is very large, particularly on the western side of the Great Basin, from which I conclude that, in addition to the visible faulting, some of the dilation must be hidden under the vast areas of late Tertiary volcanism. In particular, the rifting and volcanism in the vicinity of the Snake River may be attributed to the final phases of deformation. Here instead of the extension being distributed over a broad zone as it is elsewhere, it became very much more localised. This may simply be due to this region being so close to the pole of relative motion. If the Snake River downwarp was initiated at the time that the instantaneous pole was crossing it, then we should expect the volcanism to appear first in the west where the extension rate was highest. The time of this initial rifting would have been about 15 Myr before the present according to the model which compares favourably with a radiometric age of 13 Myr for the oldest Snake River rocks[16]. As the pole migrated further north, so the more easterly portions of the rift would have opened up and hence we can account for the observed age progression from west to east with time[16]. The other region undergoing extension at this time lay to the south of the Sierra Nevada. Assuming the Sierra Nevada to have moved with the Pacific place for say half of the total rotation, then the extension between this and the American plate would have amounted to about 400 km. The Southern Basin and Range may be traced in a NW–SE direction over a distance of about 1,200 km so here again considerable dilation is predicted. If there is sufficient evidence to show that this is too high then we may be led to assume that the motion of the Sierra Nevada with the Pacific plate occupied a smaller proportion of the total rotation between the Pacific and American plates.

The validity of the proposed mechanism will ultimately depend on how well it can explain all the large scale Tertiary tectonic phenomena in the Western Cordillera of North America. The mechanism should also be tested in other areas, particularly where palaeomagnetic data suggest that large rotations have occurred during relatively brief periods of geological time. In some instances oroclines may not be observed as they require the existence of an orogenic belt in the vicinity of the hinging poles. While linkage may perhaps be favoured in tectonic regimes where the crust has a strong grain, there does not seem to be any reason why it should not also be found to occur elsewhere.

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letters to nature

Uranus rings: an optical search

The discovery of rings surrounding Uranus[1-4] has prompted a careful re-examination of telescopic observations made nearly a year ago with a new type of solid-state area detector called a charge-coupled device or CCD. The extreme narrowness of the individual Uranus rings explains why they had not previously been detected either by visual or conventional photographic methods, for even if they were composed of highly reflecting, ice-coated particles, the rings would likely be hidden in the glare of reflected light from Uranus itself. The average reflectivity (averaged over the disk) of Uranus in the blue and green regions of the spectrum is very high, ~ 0.55–0.60. At longer wavelengths, however, methane in the planet's atmosphere begins to absorb much of the incident solar radiation, hence the bluish-green appearance of Uranus in the telescope. In the deep methane absorption band at 886 nm the reflectivity of the planet drops to only 0.012, and Uranus would thus appear very dark to any detector which was sensitive to this near-infrared region of the spectrum. It is of particular significance to the ring detection problem that such imaging would suppress the light reflected from the planet relative to that reflected from any nearby solid bodies, such as satellites or ring particles, by a factor of nearly 50. Unfortunately, most detectors have lost nearly all of their detectability at wavelengths longer than 800 nm.

The CCD is an exception. Low noise and quantum efficiencies of 0.5 and higher permit telescopic imaging of Uranus in the 886 nm methane band with acceptably short exposure times. In May 1976 we obtained several dozen images of Uranus in this band using a CCD camera at the f/13 cassegrain focus of the University of Arizona's 154 cm (Mt Lemmon) telescope. Exposure times ranged between 1 and 5 min. These images, recorded digitally on magnetic tape, have recently been re-examined to see if they contain any hint of the Uranus rings. Because the widths of the individual rings[1] lie way below the resolving power of any groundbased telescope, we can evaluate only an average reflectivity based on the simplifying assumption that all of the ring material is spread uniformly between the innermost and outermost rings—a region of space extending from 44,000 to 51,000 km from the centre of Uranus. Evidence of the rings has not been found in any of the CCD images. But it is possible to set an upper limit of approximately 0.0001 for the average reflectivity as defined above.

A preliminary examination of the reported occultation events implies that these narrow rings occupy ~2% of the space between the innermost and outermost events, and an analysis by Hubbard indicates that the individual rings themselves are only partially (approximately one-half) filled with solid particles[5]. If these numbers are accepted as being essentially correct, we find that the average reflectivity of the individual particles must be at most a few per cent, much closer to that of carbonaceous chondritic material than to the ice-coated particles in the rings surrounding Saturn[6-8]. Considering the extremely low temperatures characteristic of this remote part of the solar system (<90 K), this preliminary conclusion on the nature of the Uranus ring particles comes as somewhat of a surprise.

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Observations of Uranus occultation events

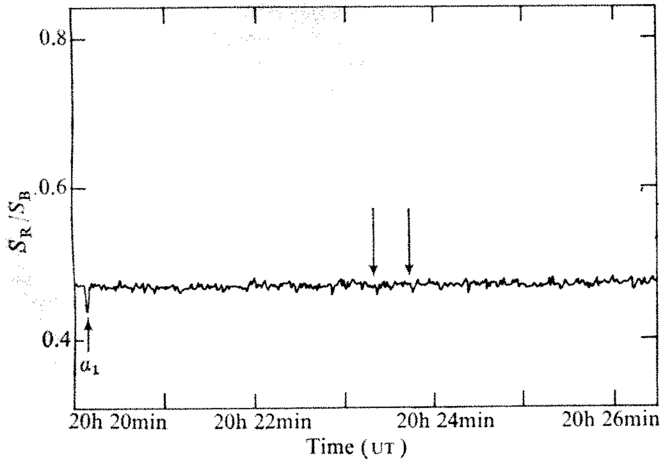
A PRELIMINARY analysis of photometric data obtained by Zellner at the Perth Observatory during the Uranus appulse to SAO 158687 on 10 March 1977 shows at least three brief occultation events which appear to coincide with portions of a Uranian ring system reported by Elliot *et al.*[1]. No occultation by Uranus itself occurred at Perth. Our group also obtained an observation by Smith with a portable telescope from the vicinity of Mauritius. The data are marginal due to variable transparency but it seems clear that there was no occultation by Uranus which diminished the stellar intensity by more than 50%. No ring events were observed from Mauritius due to the onset of a rain shower. Our observations at Perth, along with the null result from Mauritius, can be combined with other available data[2–5] to improve the deduction of the geometry of the ring features, and to obtain a preliminary upper limit to the radius of Uranus.

Our Uranus photometers recorded photon counts at 10 ms intervals on a red and a blue channel simultaneously. The red channel used an RCA C31034 photomultiplier at an effective wavelength of 8,000 Å, while the blue channel accepted light shortward of 5,000 Å, using an uncooled EMI 9789-B tube. The blue channel was used as a monitor of sky transparency and Uranian scintillation and could be used to help reject spurious 'occultation events'. In addition, both channels were chopped to the sky at a nominal frequency of 50 Hz in order to give independent measurements of sky background for point-by-point subtraction. An uncoated pellicle mirror permitted simultaneous guiding. The photon counts were recorded using a portable cassette tape recorder.

Observations at Perth, which enjoyed favourable weather, were made with the 41-cm telescope of the Western Australian University at the Perth Observatory. According to plan, data recording commenced at 20:20 UT, and shortly afterwards an occultation event of almost exactly 1s duration occurred (Fig. 1). This event was not observed by the adjacent experiment of Millis *et al.* on the 61-cm telescope which was being recentred at the time; possible subsequent low-amplitude events at 20:23:19 and 20:23:43 reported by the latter[3] are not apparent above the noise in our data, even at 0.1 s time resolution. At 21:04 UT, our observer switched to a new cassette, during which data recording was interrupted for 20 s. Observations then continued until approaching dawn terminated operations at 21:45 UT. Just before this point, two more 1 s duration occultations were observed (Fig. 2). Quite accidentally, because of different observing procedures, none of the three major events observed by Millis *et al.* were also observed by Zellner, or vice versa.

The absolute timing of our data records is established by a time signal placed at the beginning of the first cassette and at the end of the second. Final determination of the times of the occultation events is nearly complete; the preliminary times[4] already leave little doubt that the three events correspond, in the notation of Elliot *et al.*[1], to a crossing of the α-ring on the immersion side (α_1), a subsequent recrossing of the α-ring on the emersion side (α_2), followed by a crossing of the β-ring (β_2).

Fig. 1 Initial observation of the α-ring at Perth. Data have been averaged to 1 s time resolution, and the ratio of the signal in the red channel to that in the blue channel is plotted. The stellar base line is approximately at $S_R/S_B = 0.34$. Because of the narrowness of the α feature, at this time resolution the full drop is somewhat washed out. The location of putative events at 20:23:19 and 20:23:43 is indicated.



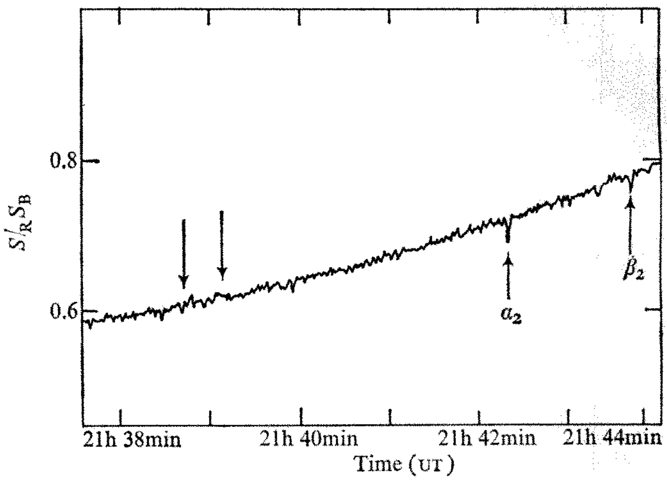


Fig. 2 Subsequent observation of the α-ring and the β-ring on the east side of Uranus. Increasing redness of the sky is caused by the approach of dawn. Possible location of rings interior to α, if they continue around the planet, is also shown.

Inspection of the data at high time resolution shows that the maximum loss of light during the α-occultations is about 50% of the steller intensity. The β-event is considerably weaker and is only marginally detected against the rapidly brightening sky. We find that the general characteristics of the α-occultations can be reproduced by a model with a randomly-placed array of occulting bodies with dimensions on the order of 1 km or smaller and an areal density ~ 50% over a band of about 10 km width. For a point source, such an array would produce intense scintillation due to diffraction. Such scintillation is not observed, nor is it expected in view of the angular size of the occulted star. Photometry by Wisniewski indicates that SAO 158687 is probably a K3III star with a projected diameter at Uranus of approximately 4.5 km, and this value has been used in the model.

We have made a preliminary fit to all timings available to us (Fig. 3), under the assumption that the rings are circular features lying in the adopted orbital plane of Uranus' satellites[6]. For each of the five confirmed rings, the coordinates of the centre of the orbit and the radius were adjusted to the best least-squares fit. We have already concluded that neither the inner four rings (α, β, γ, δ) nor the outer ring ε are truly concentric circles to within their widths. Consider two concentric circles which are crossed by an arbitrary straight line. Clearly the two line segments contained between the circles are of equal length, and will remain equal if the circles are tilted and projected to form ellipses. The relative times α–β at Perth for immersion and emersion do not satisfy this theorem, nor do many of the other relative times which are available. Small corrections for nonlinearity due to the earth's rotation etc., do not change this conclusion. Although the four inner rings are not quite concentric, and therefore time-variable, the best fits to their centres coincide to within about 100 km, and indicate a further correction[5] to the Uranus ephemeris (relative to the star) of 0.15 arc s south and 0.015 arc s west. Using the ε ring alone, the

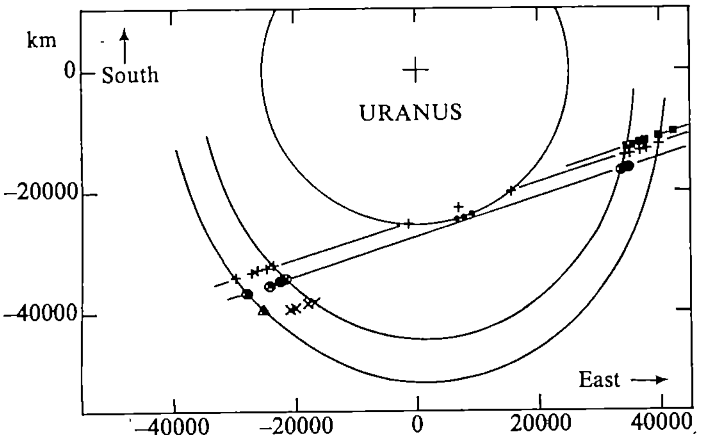


Fig. 3 Uranus occultation events. + observations of Elliot *et al.*[1–3]; ■ observations of Churms[3]; ○ observations from Perth[3,4]; ● observations from Mauritius; △ observations by Bhattacharyya and Kuppuswamy[3,4]; × observations by Mahra and Gupta.

indicated southward correction is 0.05 arc s and the westward correction is 0.015 arc s.

The degree of consistency between the ring centres can be used to locate the centre of mass of Uranus to within the same accuracy. This assumption is only valid to the extent that the limited sample of the rings from the occultation chords is not systematically affected by local ring distortions. If this is so, we then conclude that the null result at Mauritius together with the inner ring solutions places an upper limit to the radius of Uranus of 26,300 km, in opposition to a value of 27,900±500 km proposed earlier by Smith[7]. A radius as small as 25,100 km would be consistent with the ε-ring solution. We may be able to determine the radius more accurately by using the atmospheric occultations (Elliot *et al.*[1] and Churms[3]), if there is also an occultation of a few per cent in the stellar intensity at Mauritius. This effect will be sought if we can successfully correct for the effects of clouds to this accuracy.

Our group has also attempted observations from the 91-cm telescope at Hartbeespoort, South Africa, and from the 102-cm telescope at Sutherland, South Africa. We also participated in a joint experiment with the University of Cape Town on the Sutherland 188-cm telescope. None of these experiments obtained data because of heavy cloud cover at both sites.

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Wave optics of the central spot in planetary occultations

THE detection of a bright central spot[1] during the occultation of ε Geminorum by Mars[2] has demonstrated that an exponential planetary atmosphere can act as a lens with somewhat peculiar properties. In the course of a general study of certain wave-optical phenomena in planetary occultations, we have carried out an investigation into the diffraction nature of the central spot, which has not been previously considered in occultation theory. Some of the results are presented in this letter, along with implications for possible future experiments.

Assume that an observer is at a distance D from a spherical planet of radius R. The planet has a refracting, non-absorbing, exponentially stratified atmosphere with scale height H. Observations are made at wavelength λ. We assume that $(\lambda D)^{1/2} \ll H$, $\lambda \ll (HR)^{1/2} \ll D$, and $R^{3/2} \ll DH^{1/2}$. Then the planet can be regarded as a phase object[3] which modifies the phase of an incident plane wave from a distant point source by

$$\Phi_\lambda = \alpha\lambda^{-1}\exp(-r_1/H) \qquad (1)$$

where α is a constant and r_1 is the distance of closest approach of the optical path to the centre of the planet. The optical axis is then the straight line linking the centre of the planet and the distant source. Let the distance of the observer from this axis be r. Superimposing the partial waves, we calculate the intensity for $r \ll R$

$$I(r) = (2\pi)^2 \frac{RH}{D\lambda} J_0^2 \left(\frac{2\pi Rr}{D\lambda}\right) \qquad (2)$$

normalised such that the unocculted intensity $I(\infty) = 1$. The distribution of intensity resembles the Airy function and has a typical width

$$w \sim D\lambda/R \qquad (3)$$

For $w \ll r \ll R$, the average intensity goes over to the previously-derived[1,4] ray-optical result, $I \sim 2H/r$.

These results imply that, in principle, the central occultation spot could be observed to obtain very high resolution of distant objects. Assume $H = 10$ km, $D = 1$ AU, $\lambda = 5{,}000$ Å, and $R = 4{,}000$ km (Mars or Venus). The width of the central spot image of a point source is then $w \sim 2$ cm, corresponding to a theoretical resolving power of $\sim 10^{-13}$ radians (equivalent to 4 km at 1 pc). The light gathering power of the 'telescope' can be expressed in terms of an equivalent aperture R_e for a conventional telescope:

$$R_e^2 \sim I(0)\, w^2 \qquad (4)$$

We find $R_e \simeq 100$ m for this example.

Since central occultations by suitable planets are rarely seen from the Earth's surface, presumably use of the central spot technique would require the use of spacecraft to place the observer at the image of the source to be investigated. In principle, one could profitably use the technique to observe such objects as quasars, white dwarfs, and pulsars. However, certain grave practical difficulties must be mentioned:

(1) The point spread function of equation (2) is not as well-defined as the Airy function. The sharp central peak has broad wings which fall off as r^{-1}. Deconvolution of images of extended sources would thus be difficult, although objects such as binary point sources could probably be readily identified. (2) It would evidently be quite difficult to locate the image of a true point source because of its small size. (3) A real planet would be an imperfect lens. The optical axis is poorly defined for an oblate

planet, such as Mars[1]. This problem could be reduced by using a slowly rotating planet, such as Venus. Since the atmosphere will in general be turbulent, the instantaneous image may differ considerably from the ideal image shown by equation (2).

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Video tape recording of a possible ball lightning event

PHOTOGRAPHS purporting to be of ball lightning have been published by a number of authors, as discussed for example by S. Singer[1]. More recently, what is believed to be the first photograph of the formation of ball lightning has also been presented[2,3].

For the past four years I have been associated with a lightning research programme in the Transvaal Highveld region of southern Africa, in the course of which various photographic techniques have been applied routinely to the study of several aspects of the lightning discharge. Incorporated among these techniques has been the use of an automatic video tape recording system using a television camera with a silicon diode array target tube, in a manner similar to that originally reported by Winn *et al.*[4]. Particular advantages of this system in comparison with conventional photography include an enhanced sensitivity and sufficient time resolution to resolve the majority of multiple-stroke flashes. Over the past two lightning seasons some 500 lightning strokes to ground have been recorded and studied using this system.

During a thunderstorm on the evening of the 12 November 1976, a video tape recording which included a ground flash exhibiting particularly unusual characteristics was obtained. The resultant video tape was played back field by field and the individual still pictures rephotographed on a conventional display monitor. The complete event, which lasted for approximately 180 ms, is shown in Fig. 1. (The nominal time resolution of the video recording system is the time taken to sweep one field, that is, 20 ms—hence the intervals indicated in Fig. 1.) Inherent in the operation of the silicon target array is the ability to retain images of very short duration luminous events. Therefore, each still photograph constitutes an 'integrated' record—sampled at 20 ms intervals—of what is in all probability a complex process, exhibiting several stages of transient re-illumination.

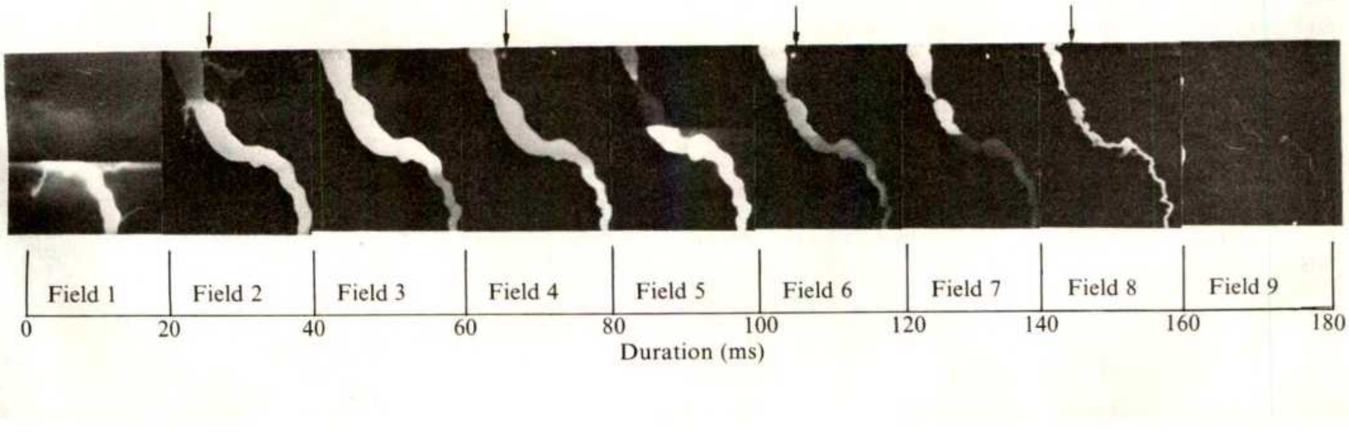
The first field in the sequence shows a faint image of a downward leader, together with the subsequent bright return stroke—which must have occurred at a time when about half the field had already been swept during the raster scan. (The 'blooming' of the very bright return stroke image is a feature of this type of camera—particularly for intense nearby strokes such as this flash, which occurred about 700 m from the camera—as determined from time-to-thunder measurements).

Thereafter, in the remaining fields of the sequence, a further three subsequent return strokes may tentatively be identified. (The main criterion for the identification of a return stroke is for a significant enhancement of channel luminosity to be apparent in relation to the images on preceding fields. Although the silicon diode camera has inherently low image persistence, remanent image persistence can occasionally make the identification of subsequent strokes rather difficult, especially in the event of short inter-stroke intervals).

Of particular interest in this sequence, however, is the recurrent appearance of a ball-shaped image in fields 2, 4, 6 and 8, apparently detached and to the right of the top portion of the main channel, except in field 2 where its position seems to coincide with that of a branch. It is interesting to observe (in Fig. 1) that the 'ball' re-illuminations seem to have occurred during the inter-stroke intervals, rather than at the times when the main discharge channel was re-illuminated by the passage of the bright return strokes. This would tend to discount the possibility of the ball being an artefact—arising as the consequence of a blemish on the lens, for example, or from a raindrop on the camera face. (No such blemish was recorded elsewhere on the video tape and no rain had occurred at the time when this flash was noted, nor for more than 10 min after the flash).

The final return stroke is followed by an indication of the persistence of a decreasing 'continuing current', as is known occasionally to occur[5] and as has frequently been recorded in the course of my measurements. However, the images of the channel in fields 8 and 9 are unusual in that they display an excessive tortuosity, together with what may be construed as 'beading'. The latter phenomenon is frequently reported to be associated with ball lightning events[1]

Fig. 1 Video-taped image sequence of multiple-stroke lightning flash showing possible appearance of ball lightning. Field 1, leader and first return stroke. Field 2, fading image of first return stroke; first appearance of ball-shaped image. Field 3, second return stroke. Field 4, fading image of second return stroke; second appearance of ball-shaped image. Field 5, third return stroke (during scan). Field 6, fading image of third return stroke; third appearance of ball-shaped image. Field 7, fourth return stroke (during scan). Field 8, persistence of continuing current ('beading'); fourth appearance of ball-shaped image. Field 9, fading of final image.



and, as has been suggested, may be produced in an analoguous mechanism[6]. Some instances of excessive tortuosity and related re-illuminated beaded processes have also been observed recently in the French research programme[7].

If one assumes that the ball image recorded here is associated with the lightning discharge, then, from a knowledge of the flash distance, the image height may be determined and is found to be approximately 300 m above ground while the apparent image diameter is about 5 m. The latter dimension, although rather large in comparison to many reputed observations of ball lightning, is not inconsistent with recent reports[2,3], but may also be the consequence of the enhanced sensitivity of the camera and the, tendency towards image 'blooming'.

It should also be noted that this particular flash struck the ground about 300 m from a 60 m high aluminium mast. This implies that at least one of the conditions was met for one class of theoretical mechanisms for the formation of ball lightning, that is, those requiring the production of intense clouds of positive space charge in the vicinity of the downward progressing negative leader for example[8], or those requiring field enhanced emission during upward interconnecting positive leader processes from grounded conductive prominences in close proximity to the descending negative leader[9].

In conclusion, however, it should be emphasised that this record is presented mainly for comment and possible interpretation. Clearly, positive identification of this event as a ball lightning event must be tentative until corroborated by further evidence. Meanwhile this observation may improve understanding of the relationship between the lightning discharge and the processes leading to the formation of ball and beaded lightning.

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Open system crystal fractionation and incompatible element variation in basalts

O'HARA[1] has recently proposed an open-system model for the fractional crystallisation of magmas involving continual replenishment of the magma chamber and only partial extraction of the fractionated liquid at each stage. This could produce a series of chemically uniform lavas (such as basalts) with very little resemblance to the primary mantle melt. It is claimed that the model can also explain large variations in the concentrations of incompatible trace elements and their inter-element ratios which would otherwise be ascribed to variable degrees of partial melting or inhomogeneity in the mantle source region. The purpose of this note is to explore the potential and limitations of O'Hara's model in this respect and to test it against the observed variability of Icelandic basalts.

I suggest that three rare earth elements (Ce, Sm and Yb) are especially useful for this purpose. In most solid–liquid equilibria relevant to basalt genesis they range from incompatible to slightly compatible with increasing atomic number. Their relative crystal–liquid partition coefficients are better understood than for many other trace elements although there is some discrepancy between experimentally determined values and estimates based on phenocryst–matrix relationships (Table 1). Lastly there is a large amount of accurate information on the concentrations of these elements in basalts, determined by mass-spectrometric isotope dilution.

The theoretical behaviour of incompatible elements during magmatic processes is reviewed by Hanson[2] to whom the reader is referred for general discussions and relevant equations. Here I have attempted to compute geologically realistic models for the significant factors in basalt magma evolution and the results are plotted in Figs 1 and 2 for Ce against Ce/Sm and Ce/Yb enrichment, relative to concentrations in the source. Brief notes on the details of each calculation follow.

- Partial Melting. An initial source composition of 55% olivine, 25% orthopyroxene, 15% clinopyroxene and 5% garnet was assumed, with these minerals entering the melt in the following relative proportions (melting modes): 10%, 20%, 40% and 30% respectively. After the disappearance of any phase (for example, garnet at 15% partial melting) the melting modes for the remaining minerals were recalculated to 100% in the same relative proportions as before. Partition coefficients used were set 1 in Table 1, since it was felt that these experimentally determined values were more appropriate to equilibrium conditions pertaining during mantle melting. Two curves are shown in the figures—*a* represents batch melting in which the melt remains in equilibrium with the residual solids, *b* is for incremental melting in which each successive 1% of the melt is removed as soon as it forms.
- Zone refining. In this process[3] melt advances by simultaneously assimilating material above it and depositing cumulates behind it, the main variable being the number of relative zone lengths processed, that is, the number of times the magma consumes its own volume. In as far as this process can be visualised at all, it must be restricted to high temperature regions of the mantle through which the basalt magma rises. Consequently the magma was assumed to be a 30% partial melt as in curve *a* above and the bulk partition coefficients for zone refining were calculated as equivalent to 60% olivine, 25% orthopyroxene and 15% clinopyroxene, using set 1 from Table 1. The results are plotted as curve *c*.
- Closed-system crystal fractionation. The parent magma was again assumed to be the 30% partial melt defined above, evolving by crystal fractionation according to the Rayleigh law. To allow for changes which would occur in major element chemistry during this process, it was assumed that the first 30% of solids formed would correspond to peridotite (50% olivine, 30% orthopyroxene and 20% clinopyroxene), the next 40% to olivine–gabbro (30% olivine, 30% clinopyroxene and 40% plagioclase) and the final 30% to anorthosite or granite (30% clinopyroxene, 70% plagioclase). The addition of quartz to the final stage has little effect on relative fractionation of these elements. For this supposed high-level process, the partition coefficients of set 2, based on phenocryst–matrix values, were preferred.
- Open-system crystal fractionation. O'Hara's basic equation (*ibid.* Fig 3) for the enrichment of a trace element in the steady state liquid contains three variables: D, the bulk partition coefficient, X, the proportion of the magma within the chamber which crystallises as cumulates and Y, the proportion of the fractionated magma which is removed and erupted a lava. The relative fractionation of two elements with similar (low) D values depends principally on the ratio X/Y, reaching effective saturation for Ce/Sm at about 10^4

Table 1 Crystal–liquid partition coefficients for basaltic melts

		Olivine	Orthopyroxene	Diopside	Augite	Garnet	Plagioclase
Set 1*	Ce	0.0010	0.003	0.098	—	0.021	—
	Sm	0.0013	0.010	0.26	—	0.22	—
	Yb	0.0015	0.049	0.28	—	4.00	—
Set 2†	Ce	0.0069	0.024	—	0.15	—	0.12
	Sm	0.0066	0.054	—	0.50	—	0.067
	Yb	0.014	0.34	—	0.62	—	0.067

*Data from experimental studies and clinopyroxene–mineral comparisons, after Hanson[3].
†Data from phenocryst–matrix studies of basaltic rocks, after Arth and Hanson[4].

and for Ce/Yb at about 10^3. At the same time, the maximum enrichment of any of these elements increases with X, but is essentially constant below $X = 0.1$. The lines plotted in the figures were thus calculated for $X = 0.01$ by varying X/Y as marked, with the effect of increasing X indicated by the dotted lines. Two cases were considered: with bulk partition coefficients corresponding to each of the first two stages in the closed-system model (that is, peridotitic and olivine–gabbro cumulates) respectively. In both a 30% partial melt was again taken as the primary magma, but for the olivine–gabbro fractionation case it was further assumed that this underwent 30% crystallisation of peridotitic cumulates before arrival at the high-level magma chamber, resulting in a fivefold previous enrichment of Ce, Sm and Yb relative to original source concentration.

The general features of Figs 1 and 2 are essentially similar and are not critically dependent on the mineralogical details of the models. The effect of the choice of partition coefficients is more significant, but it should be noted that the use of set 2 for the high-level crystal fractionation processes enhances their ability to discriminate between Ce and the other two elements. Nevertheless, partial melting is still by far the most efficient mechanism for fractionating Ce/Sm and Ce/Yb ratios, increasing these by factors of about three and 10 respectively for only a 50-fold enrichment of Ce relative to the source. Furthermore, incremental or repeated partial melting is the only process considered capable of producing a magma with lower Ce/Sm and Ce/Yb ratios than the original source material (although repeated zone refining could theoretically have a similar effect). At the other extreme closed-system crystal fractionation is scarcely at all effective at fractionating these elements with respect to each other—even less so than is normally considered for models with constant *D*s. This is because, in the third stage modelled here, removal of heavy rare earth elements in clinopyroxene is largely compensated by the preference of plagioclase for Ce. Zone refining also seems to have a negligible effect except at improbably high values for the number of zone lengths processed (>100). The curves plotted for open-system fractionation are intermediate in their efficiency at fractionating Ce/Sm and Ce/Yb ratios, but the case involving olivine–gabbro cumulates has quite low limiting values (about two times for Ce/Sm and 2.5 times for Ce/Yb).

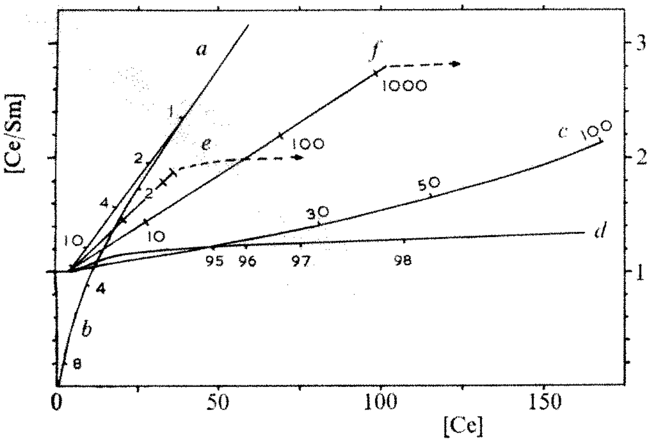

Fig. 1 Ce enrichment against increase in Ce/Sm ratio (both relative to a value of one for the mantle source) for various magmatic processes (see text for details). *a*, Equilibrium batch melting, % melt marked. *b*, Incremental melting (1% increments), % melt marked. *c*, Zone refining in the mantle, no. of relative zone lengths processed marked. *d*, Closed-system crystal fractionation, % crystallisation marked. *e*, Open-system crystal fractionation of olivine–gabbro cumulates according to O'Hara's[1] equation without assimilation, $X = 0.01$, X/Y marked; dotted portion shows effect of increasing X for $X/Y = 10^4$. *f*, As for *e* but with peridotitic cumulates.

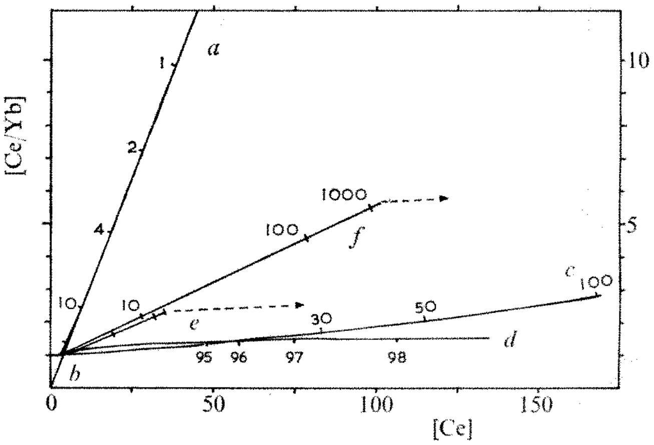

Fig. 2 Ce enrichment against increase in Ce/Yb ratio; see Fig. 1 legend for explanation.

The Icelandic basalt data against which the models are to be tested are summarised in Table 2 under four separate headings, each of which will be considered in turn. In making an initial assessment it is not necessary to know the actual abundance of Ce in the mantle source, although the reader may find it interesting to bear in mind that this is commonly estimated at somewhere between one and three times chondritic abundance.

The first grouping includes all basalts with a common ^{87}Sr/^{86}Sr ratio of 0.70315 (ref. 7), which would thus permit a common source region or parent magma. These are the majority of postglacial mainland basalts and the alkali–olivine basalts of Surtsey and Heimaey. They show a modest increase in Ce/Sm and about a threefold increase in Ce/Yb for an overall sixfold increase in Ce abundance. It is clear that the closed-system crystal fractionation model cannot explain these variations and that zone refining fails for the Ce/Yb variation (except at vanishingly low source abundances and reprocessing of several hundreds of magma volumes). At face value either partial melting or open-system crystal fractionation could be responsible. For example, batch melting of from 5% to 30% of a common source would work with original abundances about six

Table 2 Ce, Sm and Yb variations in Iceland

Grouping	Ranges*		
	Ce	Ce/Sm	Ce/Yb
I Post-glacial tholeiites and alkali basalts with constant Sr/Sr	16–93	1.0–1.6	1.2–3.8
II Reykjanes Ridge tholeiites	7–16	0.5–1.0	0.5–1.2
III All basalts	2–93	0.4–2.3	0.3–7.1
IV All volcanics	16–210	1.0–2.9	1.2–7.4

*All normalised to chondritic abundances.
Data from refs 5–7.

times chondrite. The implied source abundance would reduce to acceptable values if it were allowed that the basalts underwent some crystal fractionation of olivine on their way to the surface (as O'Hara[1] shows must be the case from consideration of their Fe/Mg ratios). Open-system fractionation as modelled here would require initial source abundances lower than those for simple partial melting by a factor of two or more, apparently a point in its favour. However, the olivine–gabbro case can easily be eliminated: in order to discriminate effectively between Ce and Sm, this process would have an even more marked discrimination between trivalent and divalent Eu as a result of the removal of plagioclase. Calculations parallel to those illustrated here show that negative Eu anomalies of at least 30% would be produced, whereas none are actually observed in any Icelandic basalt. This leaves the possibility of open-system fractionation of peridotic cumulates. In assessing whether this is a geologically feasible alternative to the previously proposed idea that these rocks result from variable degrees of partial melting of a common source[5,6], the following factors require re-emphasising. (1) The selection of partition coefficients for these models favours the open-system crystal fractionation case, perhaps unduly. (2) The proportion of magma withdrawn from the magma chamber (Y) must be exceedingly low—certainly 0.1% or less. (3) Failure to achieve the steady state postulated by O'Hara would also reduce the amount of inter-element fractionation. (4) Finally, since the absence of plagioclase fractionation restricts operation of the process to the early stages of evolution of the parent magma, possibly at considerable depths, the distinction between this and other mantle processes such as differential partial melting may be somewhat academic.

The second group shows the incompatible element variation along the Reyjanes Ridge south of Iceland (see also refs 8 and 9). Here a twofold increase in Ce/Sm (slightly more for Ce/Yb) is accompanied by little more than a twofold increase in Ce. Even equilibrium partial melting from a common source cannot account for such a rapid increase, although incremental partial melting could. When the sympathetic variation of ^{87}Sr/^{86}Sr and ^{207}Pb/^{204}Pb ratios is taken into consideration[9–11] the only reasonable explanation is one which invokes inhomogeneity in the mantle source regions, although this could well have resulted from previous extraction of partial melts, which would also explain the low chondrite-normalised Ce/Sm and Ce/Yb ratios.

Comparison with Figs 1 and 2 demonstrates that group III (all Icelandic basalts) and IV (all post-glacial volcanics, including intermediate and acid ones) must be interpreted similarly. Indeed, there is no process realistically capable of causing such high enrichments in Ce as would be implied by the derivation of Icelandic rhyolites from a mantle source which would also yield the light rare earth element-depleted basalts. The conclusion of O'Nions and Gronvold[5] that the acid rocks represent partial melts of a pre-enriched (that is, crustal) source seems well borne out by their significant negative Eu-anomalies.

O'Hara's[1] second line of argument was that if the magma chamber advanced into the oceanic crust, assimilation of basalt which had previously interacted with seawater would very much accentuate enrichment of incompatible elements and could lead to domination of the trace element and isotope chemistry by the characteristics of seawater. This could indeed explain some of the variation in ^{87}Sr/^{86}Sr ratios in, for example, island arcs. It is totally inapplicable to the case of Iceland for the following reasons. Firstly, there is no indication that seawater interaction has ever affected basalts either on the Icelandic mainland or along the Reykjanes Ridge—the observed variations in ^{87}Sr/^{86}Sr being fully confirmed by the evidence of Nd isotopes[12]. In submarine basalts from DSRP Leg 37 which do show evidence of Sr-isotope contamination[13] the Nd isotopes are unaffected[12]. Second, rare earth concentrations are little affected by seawater alteration—the contaminated Leg 37 basalts still have flat-to-light-depleted rare earth element patterns[13] and assimilation of such rocks could not account for enrichment in Ce/Sm or Ce/Yb ratios as displayed by alkali basalts from Iceland or elsewhere.

In conclusion, open-system crystal fractionation as proposed by O'Hara[1] may be a more geologically reasonable model for the modification of mantle-derived magmas than the conventional closed-system model and seems capable of producing greater variability in incompatible trace element ratios. In comparison with partial melting, however, it is severely limited in application to one of the best studied volcanic regions and cannot account for more than a small portion of the observed variation in Icelandic volcanics. In particular, it is not a viable alternative in those cases where mantle inhomogeneity has been previously invoked on the basis of trace element data. The true nature and causes of mantle inhomogeneity beneath Iceland and the Reykjanes Ridge have long been problematical[7–11,14] but, as for mid-ocean ridge segments in general, there is no escaping the need for the redistribution of incompatible trace elements, probably involving a partial melting process at some stage.

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Palaeoatmospheric argon in Rhynie chert

THE isotopic compositions and relative abundances of rare gases in the Earth's atmosphere impose rigid constraints on models of atmospheric evolution. Appreciable quantities of argon from the Devonian atmosphere have been extracted from silicified fossil plants and the isotopic composition of this argon implies that the Earth must have been strongly outgassed early in its history.

The fundamentally different origins of ^{40}Ar and ^{36}Ar in the Earth's atmosphere (radiogenic and primordial respectively) make the atmospheric (^{40}Ar/^{36}Ar) ratio a sensitive parameter for investigating the evolution of the Earth's interior. The ($^{40}Ar/^{36}Ar$) ratio in the mantle is also potentially valuable, but atmospheric contamination and the inhomogeneous distribution of potassium within the Earth make the characterisation of this component equivocal, and seriously weaken the argon degassing models which have been based on it[1,2]. The argon in the Earth's atmosphere, unlike that in the mantle, is always well mixed isotopically and represents the time-integrated product of chemical fractionation, radioactive decay and degassing processes within the Earth.

All of the ^{36}Ar in the atmosphere was originally trapped within the Earth when it was first formed. A theoretical value of 2×10^{-4} has been calculated for the ($^{40}Ar/^{36}Ar$) ratio of this primordial argon component[3] and an experimentally determined upper limit of $(1.4\pm0.6)\times10^{-3}$ has been deduced from the analyses of meteorites[4]. Almost all of the ^{40}Ar in the atmosphere (6.54×10^{19} g) is therefore the result of the decay of naturally occurring ^{40}K, the long half life of which must have effectively decoupled the entry of the two isotopes into the atmosphere. If it is assumed that ^{36}Ar was concentrated in the crust/atmosphere more readily than potassium, then the $^{40}Ar/^{36}Ar$ ratio in the atmosphere must have increased monotonically from an initial value of less than unity to the present day ratio of 295.5, the precise shape of the evolution curve depending on the variation of the argon degassing rate constants with time[5,6]. The experimental determination of the shape of this evolution curve should therefore allow argon degassing processes to be critically investigated[7].

The isolation of palaeoatmospheric argon requires the existence of geological samples which (1) are authigenic, (2) incorporated atmospheric argon during their formation, (3) are low in potassium, (4) are very old, (5) are very retentive, and (6) have had a simple geological history. Cherts are among the most suitable samples, but previous attempts to detect palaeoatmospheric argon in Precambrian cherts proved unsuccessful, possibly as a result of their marine origin and complex geological history[7]. The samples selected for analysis in this work were chips of Rhynie chert from the Lower Devonian (about 380 Myr) of Aberdeenshire, Scotland. The Rhynie chert is very low in potassium (100–150 p.p.m.), contains abundant silicified fossils of some of the oldest known land plants and is extremely well preserved[8].

The samples, weighing 50–200 mg, were analysed by the $^{39}Ar/^{40}Ar$ neutron activation method[13] so that corrections for the *in situ* decay of ^{40}K could be applied. No corrections for the neutron-induced production of ^{36}Ar and ^{39}Ar from calcium could be made, based on the ^{37}Ar, because of the long elapse time between irradiation and analysis, but the total amounts of trapped ^{36}Ar were remarkably high ($2–3\times10^{-8}$ ml STP g^{-1}) and the measured calcium content was so low (0.012%) that it could only have reduced the total ($^{40}Ar/^{36}Ar$) ratios by 0.01%, or 0.03. The total ($^{40}Ar/^{36}Ar$) ratios from two unirradiated samples (297.9 ± 1.5 and 295.7 ± 1.1) were in fact less than from the irradiated samples (303.1 ± 3.2 and 299.2 ± 0.8). The ^{36}Ar thermal release patterns from the irradiated samples (5 and 6) were bimodal, most of the ^{36}Ar being released above 1,200 °C, with maxima at 1,350 and 1,420 °C respectively. These very high extraction temperatures strongly suggest that at least the high temperature ^{36}Ar component must be occluded palaeoatmospheric argon. The release patterns for the potassium derived ^{39}Ar were broader, with maxima at slightly lower temperatures (1,220 and 1,350 °C respectively)[9].

The use of the $^{39}Ar/^{40}Ar$ method enables corrections for the ^{40}Ar produced by *in situ* decay of ^{40}K to be applied, so that the isotopic composition of the trapped component can be determined. On a correlation plot of $^{36}Ar/^{40}Ar$ against $^{39}Ar/^{40}Ar$, mixtures of trapped and potassium-derived argon define a mixing line between pure trapped argon ($^{39}Ar/^{40}Ar = 0$) and pure potassium-derived argon $^{36}Ar/^{40}Ar = 0$)[13]. The ($^{39}Ar/^{40}Ar$) ratios were so low that the ($^{40}Ar/^{36}Ar$) ratios from all gas fractions were best corrected individually for ^{40}Ar produced *in situ*, assuming an age of 380 Myr. ($J = 0.0218$, $(^{39}Ar/^{40}Ar)_K = 0.098$). The weighted mean of these corrected ratios for all gas fractions from samples 5 and 6 were (291.0 ± 1.3) and (290.9 ± 1.5) respectively, with an overall weighted mean of (291.0 ± 1.0). The weighted mean atmospheric ratio for all of the low temperature (<1,000 °C) gas fractions (291.7 ± 0.7) was indistinguishable from the total high temperature weighted mean (291.0 ± 1.6), implying that the low temperature atmospheric component may also be ancient, rather than recently absorbed, atmospheric argon.

The high temperature gas fractions are plotted on a three-isotope correlation diagram in Fig. 1. It can be seen that the 1,350 and 1,420 °C extractions from sample 6 do not fall on the mixing line within their analytical errors. Some of this discrepancy may result from calcium interference, but it should be noted that for both samples, the corrected ($^{40}Ar/^{36}Ar$) ratios increase with extraction temperature: 1,220 <1,350 <1,420 °C. This would be expected if argon extraction in the laboratory involves

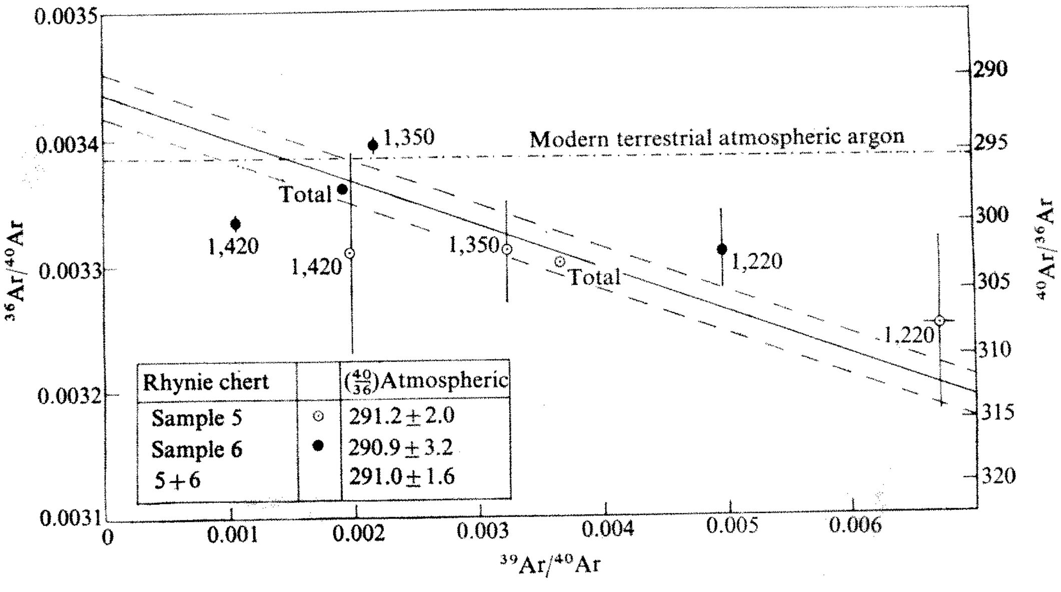

Fig. 1 Isotope correlation plot of ($^{36}Ar/^{40}Ar$) against ($^{39}Ar/^{40}Ar$) for the high temperature ($T >$ 1,000 °C) extractions from two samples of neutron-irradiated Rhynie chert (temperatures in °C). The calculated weighted mean corrected atmospheric ($^{40}Ar/^{36}Ar$) ratio is 291.0 ± 1.6 assuming a gas retention age of 380 Myr. The points marked 'total' represent the arithmetic sums of the high temperature points from each sample and they both fall slightly below the mixing line because the points have not been individually weighted according to their analytical errors.

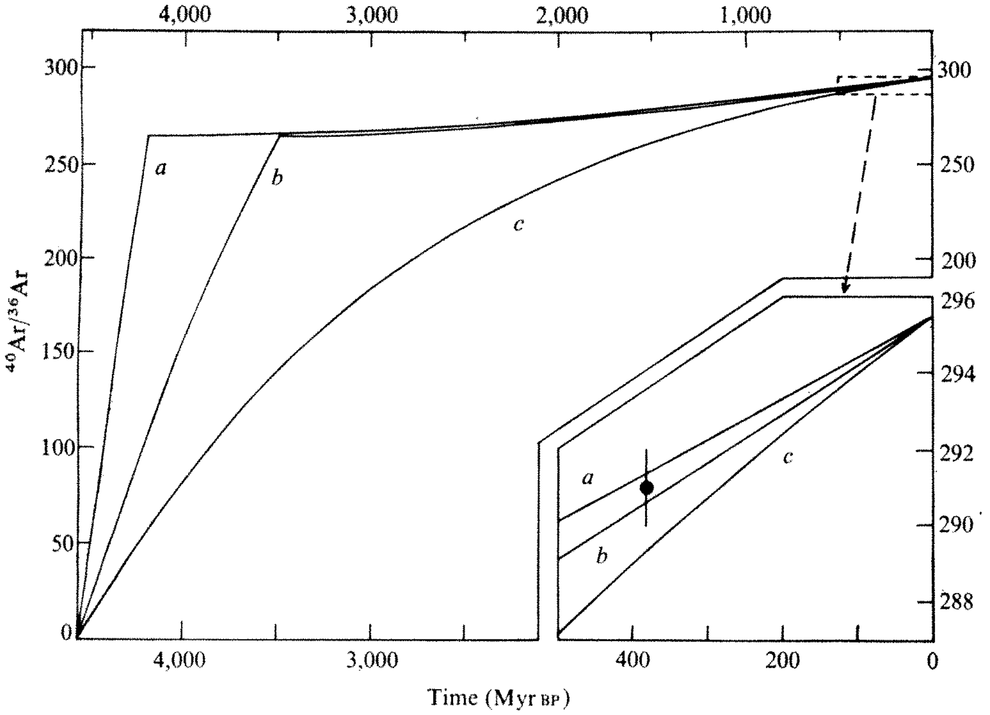

Fig. 2 Evolution curves for the ($^{40}Ar/^{36}Ar$) ratio in the Earth's atmosphere assuming total loss of ^{36}Ar to the atmosphere 4.55×10^9 yr ago. *a*, *b*, Two stage degassing models delimiting a range in potassium content for the Earth from 350–140 p.p.m. and catastrophic degassing periods before 4.2×10^9 and 3.5×10^9 yr ago respectively. *c*, Upper limiting case for single stage degassing models, corresponding to $\alpha \gg \lambda_{40}$ and a potassium content for the Earth of only 75 p.p.m.

mass-dependent diffusion. In these conditions the lighter isotope, ^{36}Ar, may be released more readily than the heavier isotope, ^{40}Ar, giving rise to artificially low initial ($^{40}Ar/^{36}Ar$) ratios. The concordance of the palaeoatmospheric ($^{40}Ar/^{36}Ar$) ratios from the two samples argues against geological diffusive fractionation and both effects could be critically investigated by measuring ($^{38}Ar/^{36}Ar$) ratios in larger, unirradiated samples. But even without the determination of the isotopic ratio of the trapped component, the fact that large amounts of ^{36}Ar are so strongly bound within the sample is in itself a very significant finding.

An atmospheric ($^{40}Ar/^{36}Ar$) ratio of (291.0 ± 1.0) 380 Myr ago imposes a firm constraint on argon degassing models of the Earth. This value is higher than those predicted by all first order degassing models[5,7], unless it is postulated that ^{36}Ar is retained in the mantle preferentially to potassium. The degassing rate constant necessary to account for all of the ^{40}Ar in the present day atmosphere depends on the potassium content of the Earth. The most extreme first order model, for example, predicts a ($^{40}Ar/^{36}Ar$) ratio as high at 289.0, 380 Myr ago but requires a potassium content for the Earth of only 75 p.p.m. (Fig. 2). To explain the slightly higher measured ratio, the degassing constant must have decreased as a function of time. The simplest model consists of two stages, during the first of which the degassing constant was large compared with the decay constant of ^{40}K, as in the extreme single-stage first-order model. Since the termination of this continuous degassing period, the degassing constant has been small. Other simple models might invoke a degassing constant which has decreased linearly, or exponentially, with time. There exists a family of possible two-stage evolution curves for the atmospheric ($^{40}Ar/^{36}Ar$) ratio, depending on what value is taken for the potassium content of the Earth (Fig. 2). This is not known precisely but is constrained by the measured global heat flow. It could be as high as the chondritic value of 880 p.p.m., but the uranium-to-potassium ratios in a wide range of terrestial rocks are remarkably constant (about 10^{-4}) and significantly higher than in chondritic meteorites[10], implying a potassium content of between 250 p.p.m. and 165 p.p.m., or even less for an initially hot Earth[11]. This range in potassium content corresponds to a continuous degassing period which terminated between 4,000 and 3,700 Myr ago. The uncertainty in this time which arises from the error in the measured atmospheric ratio ($< \pm 40$ Myr) is minor compared with that arising from the uncertainty in the potassium content of the Earth and from the limitations inherent in the simplified model. The two-stage evolution curves closely follow one another during the second stage and therefore the determination of the palaeoatmospheric ($^{40}Ar/^{36}Ar$) ratio in suitable Precambrian samples would provide a crucial test for the model.

The determination of the ($^{40}Ar/^{36}Ar$) ratios in the Devonian atmosphere provides further evidence for the early catastrophic degassing of the Earth[14]. Thermal history calculations[11] and lead isotope studies[12] imply early chemical differentiation and crust formation. This crust must then have remained open with respect to argon for about 700 Myr after the formation of the Earth, a process which may have been facilitated by the same intense meteoritic bombardment to which the moon was subjected, a bombardment which terminated about 3,850 Myr ago. The continuous degassing period suggested by this work is certainly consistent with the absence of terrestial rocks with known crystallisation ages much in excess of 3,750 Myr (ref. 15).

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Lead isotope measurements from the oldest recognised Lewisian gneisses of north-west Scotland

No isotope ages exceeding ~2,800 Myr have yet been reported from Lewisian gneisses. Furthermore, there is no published isotopic evidence suggesting that Lewisian gneisses incorporate a significant proportion of much older, re-worked sialic crust. Recently, Davies[1] has interpreted field evidence from small, discordant amphibolite bodies as indicating a pre-2,800-Myr history of the Lewisian Complex in the type localities of Scourie and Laxford, Sutherland, which is directly comparable with that of the Godthaab area of West Greenland. He has proposed that equivalents of the ~3,700-Myr-old Amîtsoq gneisses and ~2,800-Myr-old Nûk gneisses can be distinguished, on the tentative assumption that the basic bodies are the equivalents of the Ameralik dykes[2–4]. This paper tests this hypothesis using Pb isotopes.

In Table 1, we present 12 whole rock Pb isotope analyses on the Older ('Uamhaig') gneisses of Davies[1], as well as eight analyses on other nearby granulite gneisses within the type Scourian Complex[5]. The Older gneiss samples were supplied by, or collected in collaboration with, Dr F. B. Davies. Almost all analysed samples are one- or two-pyroxene quartzofeldspathic gneisses, some of which exhibit incipient retrogression into amphibolite facies. Several samples contain hornblende, garnet or biotite.

Pb was extracted from whole rock powders by the dissolution-electrodeposition procedure of Arden and Gale[6]. Blanks were between ~0.1 and 0.5% of total Pb extracted, averaging 10 ng per dissolution. Isotopic analyses were made on a 12-inch solid-source mass spectrometer, using silica gel–phosphoric acid activator on single Re filaments. Decay constants for ^{238}U and ^{235}U were taken as 1.551×10^{-10} yr^{-1} and 9.85×10^{-10} yr^{-1} respectively[7]. (Previous publications from this laboratory used the older recommended values of 1.537×10^{-10} yr^{-1} and 9.72×10^{-10} yr^{-1}).

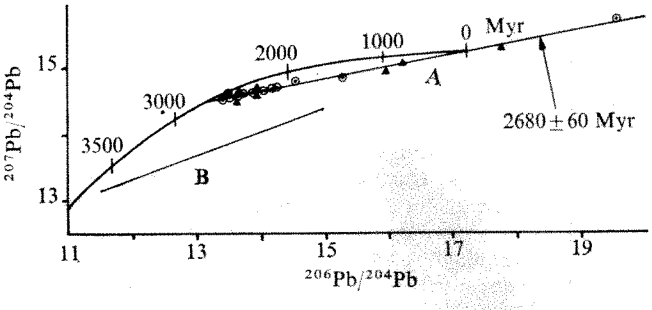

Fig. 1 Pb/Pb plot of analytical data (line A). ⊙, Older gneiss group of Davies[1]; ▲, Scourie gneisses. For comparison, line B is the maximum range of present-day measured values for ~3,700-Myr-old Amitsoq gneisses of West Greenland.

^{207}Pb/^{204}Pb and ^{206}Pb/^{204}Pb ratios are plotted in Fig. 1, line A. The data points define an isochron, but to achieve perfect fit analytical errors must be magnified by three. The isochron yields an age of 2860±60 Myr (2σ), which agrees well with other recent age determinations on Scourian rocks[8–10]. The ^{238}U/^{204}Pb ratio (μ_1) for the source region of the gneisses was 7.7—the value taken for plotting the Pb isotope primary growth curve in Fig. 1. This value is well within the range typical of upper mantle source regions. There is also a good correlation (not plotted) between ^{208}Pb/^{204}Pb and ^{206}Pb/^{204}Pb data, yielding a value of 3.1 for the Th/U ratio of the gneisses (correlation coefficient = 0.97). This is within the characteristic range for many primary igneous rocks.

For comparison, line B in Fig. 1 represents the maximum range of measured present-day Pb isotope compositions for ~3,700-Myr-old Amîtsoq gneisses of West Greenland (ref. 11, 12 and unpublished data) which include the most unradiogenic terrestrial Pb isotope ratios yet reported. It is evident that the analysed gneisses (Table 1) contain no detectable Pb derived from a source similar to Amîtsoq gneisses. At 2,800 Myr ago, Pb in Amîtsoq gneisses was even less radiogenic than today and nowhere near the initial Pb isotope composition of any known Lewisian gneisses (Fig. 1). We believe that this argues strongly against the

Table 1 Analytical data

Sample no.	Locality and grid reference	^{206}Pb/^{204}Pb*	^{207}Pb/^{204}Pb*	^{208}Pb/^{204}Pb*
	Older gneiss group ('Uamhaig' gneisses[1])			
CD1	Badcall Bay, 146418	13.85	14.63	33.86
CD2	Badcall Bay, 146418	19.54	15.73	38.50
CD3	Scourie More, 142442	13.41	14.53	33.15
CD4	Scourie More, 142442	13.48	14.60	33.38
D1	Badcall, 145417	14.82	14.80	35.21
D3	West Scourie More, 140448	14.02	14.65	33.64
D4	Badcall, 145418	15.27	14.85	35.12
D5	West Scourie More, 139448	13.63	14.58	33.24
D7	Upper Badcall, 184443	14.23	14.71	34.33
D12	North Badcall, 152432	14.14	14.70	34.44
CM 1A	Badcall Bay, 146420	13.49	14.58	33.55
CM 1B	Badcall Bay, 146420	13.69	14.63	33.89
	Scourie gneisses			
MW 13	Upper Badcall, 161432	15.94	14.96	36.72
MW 14	Upper Badcall, 155417	13.92	14.67	34.09
MW 17	West Scourie More, 145447	16.19	15.05	35.88
MW 20	North-west Scourie More, 142448	13.67	14.60	33.57
MW 22	Scourie Bay, 149449	13.89	14.62	33.92
MW 38	Scourie Bay, 155449	13.89	14.64	33.86
MW 40	Scourie Bay, 152452	13.60	14.50	33.42
MW 41	Scourie Bay, 152452	17.76	15.30	37.05

*Average within-run precision better than 0.1%.

presence of such ancient sialic crust within, or beneath, this part of north-west Scotland. In agreement with earlier work[13] we postulate that the presumed igneous precursors of Scourian gneisses separated from upper mantle or basic lithosphere not more than ~100–200 Myr before the measured date.

Whilst these findings do not necessarily contradict the field evidence of Davies[1] regarding the relative ages of different gneiss groups, they suggest a much more condensed time sequence possibly similar to that of the Nûk gneisses of West Greenland[4] and render detailed Archaean correlation between north-west Scotland and West Greenland implausible.

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Giant late Jurassic sabkhas of Arabian Tethys

THE discovery that anhydrite–gypsusm evaporites with characteristic textures are forming today in the shallow subsurface of supratidal coastal sabkhas and in continental sabkhas from Arabia[1,2] has revolutionised the interpretation of ancient evaporite sucessions. Several ancient evaporite basins, of great economic importance, lately regarded as former hypersaline lagoons or barred basins, have been recently re-interpreted as the deposits of former prograding sabkha plains[3,4]. In this note we demonstrate that late-Jurassic evaporitic sulphates and minor chlorides of Saudi Arabia, Iran, Iraq, Kuwait, Trucial States, Yemen and Aden were deposited on giant sabkhas ($>2\times10^6$ km²) on the southern margin of the former Tethys Ocean. At their maximum extent in Tithonian times these inferred sabkhas seem to have been among the largest yet discovered. The evaporitic facies throw some light on the evolution of giant sabkha plains. In addition the sabkha plains form the caprocks to some of the world's most prolific oil reservoirs[5–7], so that understanding of evaporite genesis is of outstanding economic interest for Middle East stratigraphic studies.

In Saudi Arabia (Figs 1, 2), anhydrite members occur at four main horizons in the Arab and Hith Formations of late Kimmeridgian to Tithonian age[5,6]. The uppermost Hith anhydrite occurs in Trucial Coast and Iranian subcrops[8–10] and finds correlatives in the Gottnia anhydrite of Iraq, Kuwait and north-west Iran[7,8,11] (Figs 1,2). The Arab-Hith Formations have been regarded as slightly younger than the Amran Group evaporites of Yemen and Aden[9] but these latter are poorly dated, being overlain conformably by the Upper Tithonian Marine limestones. Faunal data in ref. 9 cannot rule out the possibility that the Amran evaporites may be late Kimmeridigian to middle Tithonian in age.

Investigations of the Arab–Hith evaporites in the Saudi surface outcrops are hindered by massive Tertiary dissolution, while in the subcrop there are, as yet, only generalised thickness data from oil exploration coreholes[12]. At Dahl Hith, however, 32 km south-east of Riyadh, the type section of the Hith anhydrite occurs (Fig. 2) in a deep dissolution cavern where we have recently documented clear evidence that sabkha-type facies are present, with nodular, 'chicken-mesh' aggregates and enterolithic veins in quartz silt-rich, dolomitised grainstone hosts. Crudely layered anhydrite units with disrupted fabrics, resedimented clasts and laterally persistent anhydrite–dolomite varves also occur. The Arab-A carbonate member consists of dolomitised pelletal grainstones showing traces of large scale cross stratification. The Arab–B evaporite member at the base of the section shows particularly fine nodular and layered anhydrite. At outcrop, sections in the basal Arab-D evaporites are rare in the collapse breccia zone, but at Wadi Nisah (Fig. 1) we have found bedded remnants of chicken-mesh anhydrite, now calcitised, in a dedolomitic calcite matrix. Traced eastwards in the subsurface, the Arab

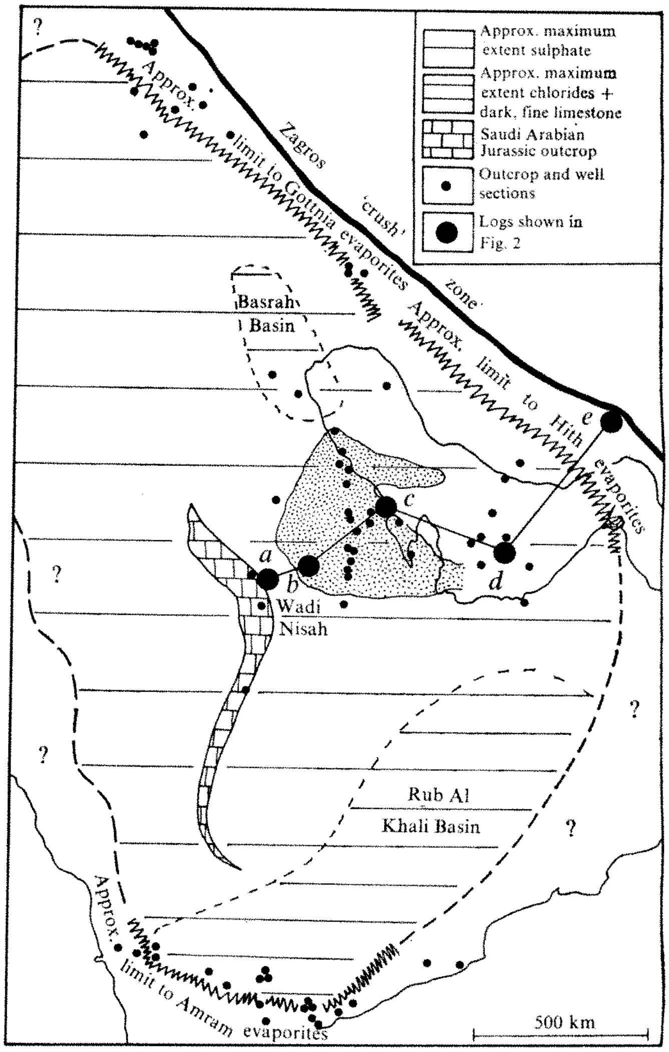
?
Approx. limit to Gottnia evaporites
Zagros 'crush' zone
Approx. limit to Hith evaporites
Approx. maximum extent sulphate
Approx. maximum extent chlorides + dark, fine limestone
Saudi Arabian Jurassic outcrop
Outcrop and well sections
Logs shown in Fig. 2
Basrah Basin
a
b
c
d
e
Wadi Nisah
?
?
?
Rub Al Khali Basin
?
Approx. limit to Amram evaporites
500 km

Fig. 1 Map to show principal surface and subsurface sections used to delimit the approximate near-maximum extent of the Arabian late Jurassic sabkhas during Hith Anhydrite times (after data in refs 5–12, 16–19 and unpublished data). Stippled area indicates maximum development of porous lime sands during Arab-D times[16] (see Fig. 2). These now form the major Saudi oil reservoirs.

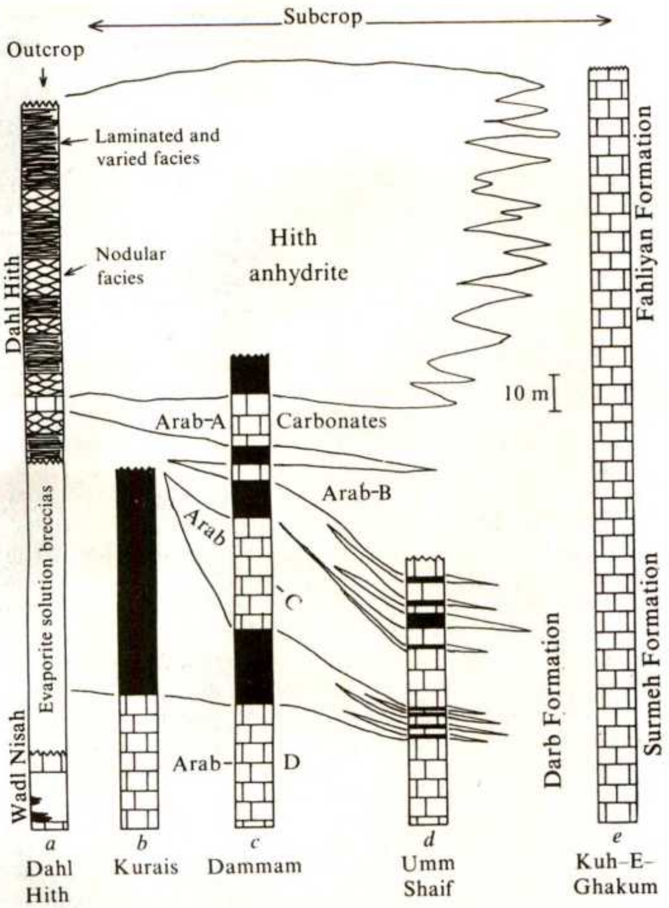

Fig. 2 Major facies changes in the Arab/Hith Formations and their correlatives in the Gulf and Iran to show general eastwards tongueing-out of evaporites away from the landward parts of the supposed sabkhas in central Arabia. Location of sections in Fig. 1. (Data for *a*, authors' results; *b*, *c*, refs 5, 6, 12; *d*, ref. 10; *e*, ref. 8). Note that anhydrite in section *d* is of nodular type only.

Formation anhydrite members thin progressively towards the Persian Gulf while the interbedded grainstones of presumed offshore bar origin tend to thicken[5,6,12] (Fig. 2) until they thin on to the Surmeh shelf of the south-eastern Gulf and Iran[8]. In the Umm Shaif field of offshore Abu Dhabi the Dharb Formation shows many thin sabkha cycles and is correlated with part of the Arab Formation in the Saudi and Qatar subcrops[10]. As the Hith anhydrite is traced eastwards into Iran it is replaced by partly dolomitised offshore marine carbonates of the Surmeh Group[8] (Fig. 2).

From the above discussion it would seem that sabkha progradation occurred repeatedly from west to east during Upper Kimmeridgian and Tithonian times, that is, from the core of the Arabian shield towards the shelf margin of Arabian Tethys (>1,200 km). Sabkha progradation was followed in each depositional cycle by marine transgression. Sections in the present westernmost outcrops thus contain evaporitic facies precipitated progressively further from the Tethyan coast as time proceeded during each progradational episode. Following Walther's Law, therefore, the vertical facies sequence within each evaporite cycle should demonstrate how evaporitic environments evolved with time. In eastern areas the thin sabkha cycles such as seen in the Umm Shaif field[10] represent more or less precise analogues to the deposits of modern coastal sabkhas which have only been developing since the post-glacial standstill some 4,000–5,000 years BP (ref. 2). In this area repeated transgression followed by carbonate deposition and then sabkha progradation has given rise to many thin sabkha cycles. To the west, however, in the outcrops of the Arab–Hith Formations at Dahl Hith, there are repeated thicker rhythms of nodular anhydrite, sometimes with signs of a carbonate matrix, followed by laminated and varved anhydrite. It is very noticeable that the carbonate members, dominant in the Arabian–Trucial States coastal areas, thin progressively westwards as well (Fig. 2). Sabkha cycles with hitherto unexplained layered and varved upper members are also recorded from the Permian of Cumbria, England[13].

We infer that as coastal sabkhas prograde out to some critical width subsurface anhydrite nodule growth ceases and the interior sabkha plains are converted in some way into periodically emergent playas giving rise to laminated and varved anhydrite. Exactly how this process occurs is at present unclear. The evidence of resedimented anhydrite clasts, and buckled and broken anhydrite laminae indicate that the postulated brine pools were frequently dried up. Scattered quartz grains may indicate some aeolian input into the sabkha plains but we stress that the more important features of continental sabkhas as discussed by Kinsman[2], such as remnant aeolian dune sets, deflation surfaces and adhesion ripples are absent. As Kinsman has noted[2], a broad prograding sabkha surface will tend to be depressed below the base level of erosion as basin downwarp continues. When, as in the present case, there is an absence of aeolian deposition a broad depression would result which might receive brine input due to (1) seepage from the local brine table, (2) marine recharge, possibly from channel courses linked seawards to marine areas, (3) surface drainage from continental hinterlands, (4) continental brine migration, likely to be most important relatively close to the landward sabkha margin[14]. The source of such brines is clearly of the utmost importance and deserves further study. In such interior parts of giant sabkha plains sodium- and potasssium-rich bitterns should also occur, their formation being aided by the fractionation process outlined by Smith[15] for the Permian Zechstein evaporites. Potash is not known in the Arabian evaporites, but extensive halite occurs in Kuwait[7], Aden[9] and the Saudi Rub al'Khali[16] basin. These areas of chloride precipitation, not usually furthest from the presumed Tethyan shoreline, may represent areas of the Arabian platform undergoing greatest subsidence[16]. Associated organic-rich, sometimes foetid micritic limestones lack the well-washed skeletal constituents of the typical Arab Formation carbonates[16] and may be ascribed to rapidly subsiding, possibly partly euxinic, brine-filled basinal areas within the Arabian platform.

As Kinsman[2] has pointed out, the rate of shoreline regression in the modern Trucial Coast, has been about 1–2 m yr^{-1} during the past 4,000–5,000 years. Over 10^6 years a sabkha could develop 1,000–2,000 km wide, as postulated above. A minimum of four periods of major late Jurassic sabkha progradation is recorded in the Arab/Hith Formations in the western Saudi areas. These deposits together probably formed over a total of about 10^7 yr, so it would be entirely in order for individual sabkha progradation to have occurred over periods of about 2×10^6 yr.

Our palaeogeographical sketch (Fig. 3) shows the maximum likely extent of the giant Arabian sabkhas in relation to a late-Jurassic restoration of the continental plates that formerly bounded the Tethyan ocean. It is clear that the coastal plain was bounded on three sides by areas undergoing normal shallow marine carbonate and clastic sedimentation. Repeated marine transgression over the structurally simple Arabian platform, giving rise to sourcerocks and potential oil reservoirs, was thus followed at least four times by massive eastward coastal progradation and sabkha evaporite formation during late Jurassic times. Detailed sedimentological analysis of these evaporites will follow in a later contribution.

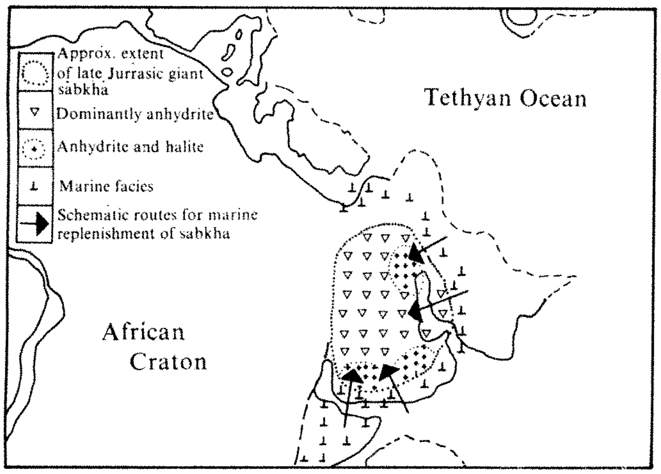
Approx. extent of late Jurrasic giant sabkha
Dominantly anhydrite
Anhydrite and halite
Marine facies
Schematic routes for marine replenishment of sabkha
Tethyan Ocean
African Craton

Fig. 3 Maximum likely extent of the Arabian giant sabkha for Hith evaporite times in relation to the distribution of late-Jurassic marine facies on the southern margin of the Tethys ocean. We do not indicate a separate Iranian plate on this sketch. Plate configuration after ref. 20. Additional facies data for East Africa and eastern Mediterranean from refs 21, 22. Position of replenishment arrows entirely schematic.

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Contact angle and film jumps

THE mechanism of capillary rise in a random sphere packing is such that, depending on the absolute value of the wetting- or contact-angle, θ, at equilibrium either a saturation gradient, widest for $\theta = 0$, is observed, or a sharp front between the saturated and the dry part of the porous medium, above some critical value of θ (ref. 1). We present here another partition in the phenomenological description of capillary rise for systems which have $\theta > 0$. There are systems in which θ remains constant and others in which θ decreases during capillary rise. In particular, attention is drawn to an uncommon hypothesis that can explain a decrease in θ. Given the nature of the absorption isotherm in the neighbourhood of saturation, small variations in vapour pressure may induce discontinuous film thickness jumps on a microscopic scale, which may display itself macroscopically as a changing contact angle.

Only capillary rise of organic liquids in random packings of glass beads is considered. For capillary rise of a sharp liquid front in a packing[3]

$$\mathrm{d}h/\mathrm{d}t = (k/h)(h_c - h) \qquad (1)$$

where h is the height of capillary rise at time t, h_c the (capillarity) driving force, and k the Kozeny–Carman permeability. The value of k depends only on the packing and the viscosity and the density of the liquid. The value of h_c depends on the geometry of the pore space, the liquid surface tension, σ, and the contact angle. Hence, for a combination of a solid packing and a liquid, a straight line should be observed when $\mathrm{d}h/\mathrm{d}t$ is plotted against $1/h$—that is, the velocity curve is straight. The value of k can be calculated for random sphere packings. The value of h_c can then be calculated from equation (1). In our experiments[4] the emphasis was on the variation of many practical relevant parameters, not on the purity or cleanliness of the substances. Capillary rise was observed for *n*-hexane, *n*-heptane, paraffin oil (medicinal), toluene, ethanol, *n*-octanol, glycol, acetone, carbon tetrachloride. α-bromonaphthaline and dichloroacetic acid. Glass spheres of different chemical constitution were used. For some glass surfaces and some liquids θ seemed to be zero. In that case a saturation gradient is formed at equilibrium[1,5]. Before the saturation gradient is formed, equation (1) is obeyed in that the velocity curve is linear and k agrees with theory, such behaviour is called rapid capillary rise. When $\theta \neq 0$ equation (1) is rarely obeyed. An example is given in Fig. 1 for paraffin oil; when $\theta \gg 0$ no saturation gradient is formed at equilibrium[1,5].

When the velocity curve is concave towards the $\mathrm{d}h/\mathrm{d}t$ axis, the $\mathrm{d}h/\mathrm{d}t$ change is slower than equation (1) predicts. This is called slow capillary rise. (Hence 'slow' and 'rapid' do not refer to the absolute value of the capillary rise. A slowly moving viscous liquid such as paraffin oil displays rapid rise. A rapidly moving thin liquid such as hexane may display slow rise, see Fig. 1. Slow capillary rise has been observed for all liquids mentioned above and at some glass surfaces.

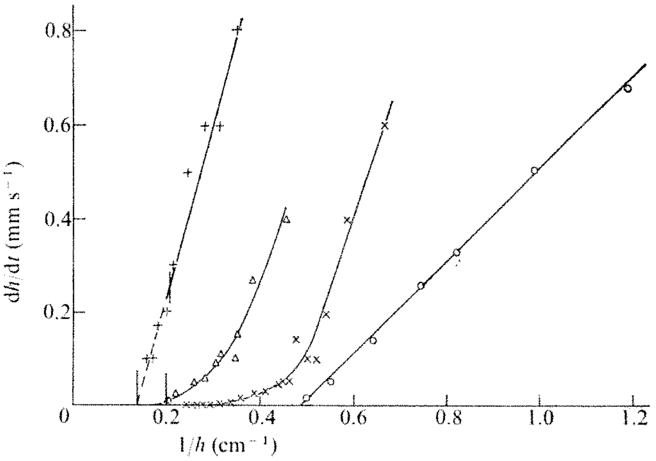

Fig. 1 Capillary rise experiments are carried out by submerging a packing of glass beads 2 mm below a liquid surface, which is kept at a constant level. The packing is contained by a glass tube and supported by a glass filter, or it is slightly sintered, in which case no support is needed. The packing is prepared using a controlled deposition and vibration technique. The homogeneity is checked by means of X-ray absorption. The capillary rise is observed visually or by X-rays. When there is a sharp liquid front both methods give the same result. The packing, X-ray absorption, and sintering techniques have been described in detail elsewhere[2]. The capillary rise of hexane +, △, ×, and paraffin oil ○ in a packing of 300–400 μm sodaglass beads is depicted: ○ and +, rapid rise; △ and ×, slow rise; + and △, saturation gradient at equilibrium; ○, sharp front at equilibrium (coordinates on vertical scale have to be multiplied by 100).

Reports elsewhere support the fact that slow capillary rise corresponds to a decreasing contact angle during capillary rise[4]. Although equation (1) applies formally in that the movement of the liquid front is caused by the pressure gradient $(h_c - h)/h$ and constant resistance to flow, k, the observed dh/dt is determined by the change in h_c. We carried out many experiments to check that the slow capillary rise was not due to secondary factors such as a change in packing structure, air entrapment, capillary condensation, or evaporation. Becuase the geometry is constant, for any liquid k is constant and h_c depends only on the contact angle and the liquid surface tension, σ. Further, it seems impossible that slow rise is due to an increasing σ (θ being constant), because this would imply for the curves of hexane (which has $\sigma = 18$ dyn cm^{-1}) that at the beginning of capillary rise σ is about 8 dyn cm^{-1}.

It is not possible to discuss here the numerous hypotheses that might explain a decreasing θ. The following possibilities were considered: unknown compounds on the solid surface, in the liquid, from the air, etc.; heterogeneity of the surface (geometric or energetic); (electro) chemical changes of the surface (penetration, solution); change in film properties (formation, displacement, (selective) evaporation); capillary condensation. It is almost impossible to design experiments that discriminate between these hypotheses. For example, with toluene as a wetting liquid, *inter alia*, the following parameters were varied—glass composition: lead–, soda–, boro–, silicate–, and aluminosilicate–glass; numerous treatments, including vacuum, heat, acids (also hydrogen fluoride), NaOH, detergents; various rinsing and drying methods (including those which yield $\theta = 0$ for water on glass plates); liquid: from different suppliers, degassed, freshly distilled, dried over molecular sieves, dissolved compounds added of higher and lower surface tension and or vapour pressure; vapour: in the absence of air, evaporating from liquid surface, with a gas pressure opposing capillary rise [$(h_c - h - \Delta P)$ instead of $(h_c - h)$ in equation (1)].

Although these variables sometimes had a small effect on the kinetics of rise, in no case was rapid rise observed, in the sense of obeying equation (1). Moreover, an increasing contact angle was never observed (This would be shown by k being higher than the theoretical value). Glass surfaces yielding $\theta = 0$, could be changed (irreversibly) into surfaces yielding $\theta > 0$ and slow rise, by applying surface treatment. Therefore, it is concluded that none of the hypotheses receives significant support. A more interesting but more speculative hypothesis to explain the general phenomena of decreasing contact angles follows.

When $\theta = 0$, at equilibrium, after capillary rise, the adsorbed film, a thin macroscopic wetting film, and the meniscus are in equilibrium. When $\theta > 0$ there is some kind of discontinuity between the bulk liquid and the adsorbed film. Although straightforward and mentioned by Freundlich[6] in 1922, it is seldom realised that, if $\theta \neq 0$, the adsorption isotherm should cut the $\varphi = 1$ axis one or more times (φ is the relative vapour pressure, p_v/p_s).

One way to describe the nature of the adsorption film in the region $\varphi = 1$ is by using the concept of disjoining pressure[7], p_d, defined as $l\,d\mu/dl$, with l the thickness of the film (defined for molecular as well as macroscopic films) and μ the chemical potential per unit volume of adsorbed film. The film is thermodynamically stable if $(\partial\mu/\partial l)_T > 0$. If p_d is positive for all l, $\theta = 0$ for all values of l. If $\theta > 0$, there is a region of unstable film thickness for which p_d is negative. This means that at some vapour pressures, in particular at p_s, different θ, corresponding to different l are possible. Hence, small variations in p_v (or μ for a non-homogeneous multicomponent system) may cause discontinuous changes in l, called film jumps.

Many strange phenomena become more intelligible when it is realised that variations in vapour pressure may cause discontinuous jumps in the film thickness and hence the contact angle. The best examples are found amongst the experiments of Bangham *et al.*[8]— for example θ for a drop of benzene placed on a micasurface decreased when brought under the influence of a jet of supersaturated benzene vapour. Further, it seems possible that some Marangoni phenomena in which a solid surface is present are in fact caused by film jumps.

During capillary rise small fluctuations in temperature (or concentration of unknown compounds) cause local changes in vapour pressure (or thermodynamic film potential) and therefore possibly film jumps, and hence a change in θ. This causes a minor local increase in capillary rise, which may trigger the nearest cavity (or 'pore', refs 1, 5) to be filled, which gives a minor rise of the whole liquid–vapour interphase. A local increase of θ, will not decrease the macroscopic height of the liquid front, because the curvature at which a cavity drains is much higher than the curvature at which it fills (structural hysteresis). This may explain why until now we have not observed slow rise in cylindrical capillaries. (Not many data are available, however, because nearly always $\theta = 0$.) The fact that paraffin oil does not show the behaviour described may correspond to the observation by Hardy[9] that there is almost no adsorption film in this case.

Although we realise that we have not given very strong support for the film jump hypothesis, we think it is worth consideration. Wetting phenomena have some role or other in almost any scientific discipline. We would appreciate any information about other phenomena or considerations that have a bearing on this subject.

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Palaeoecological investigations of a site at Hampstead Heath, London

OUR understanding of the vegetational history of Britain is based mainly on studies of peat-bogs, lake and kettlehole infills[1]: away from areas where such deposits exist, there is a paucity of information[2]. A multidisciplinary investigation of a deposit associated with a Mesolithic site on Hampstead Heath, North London, is providing rare evidence about man's effect on the vegetational succession of south-east England. The pollen spectrum indicates that *Tilia* rich forest existed until the elm decline, followed by continuing clearance episodes and heath formation. Seeds and beetle remains, which provide more precise information on local conditions, confirm the overall pattern of change from natural forest to cleared areas used for cultivation and grazing. The Mesolithic site of West Heath, Hampstead (TQ2566 8676), is being excavated by the Hendon and District Archaeological Society under the direction of Mr D. Collins[3]. The site stands on dry, acid Bagshot Sands, but environmental investigations have been carried out at West Heath Spa, a boggy area about 300 m to the south-east, where permanent waterlogging is maintained by a chalybeate spring. At this spot, samples for pollen, seed and insect analyses were collected at 5-cm intervals from below the modern root layer to a depth of 130 cm. Results of these studies are briefly described here.

A pollen diagram (Fig. 1) has been drawn up from

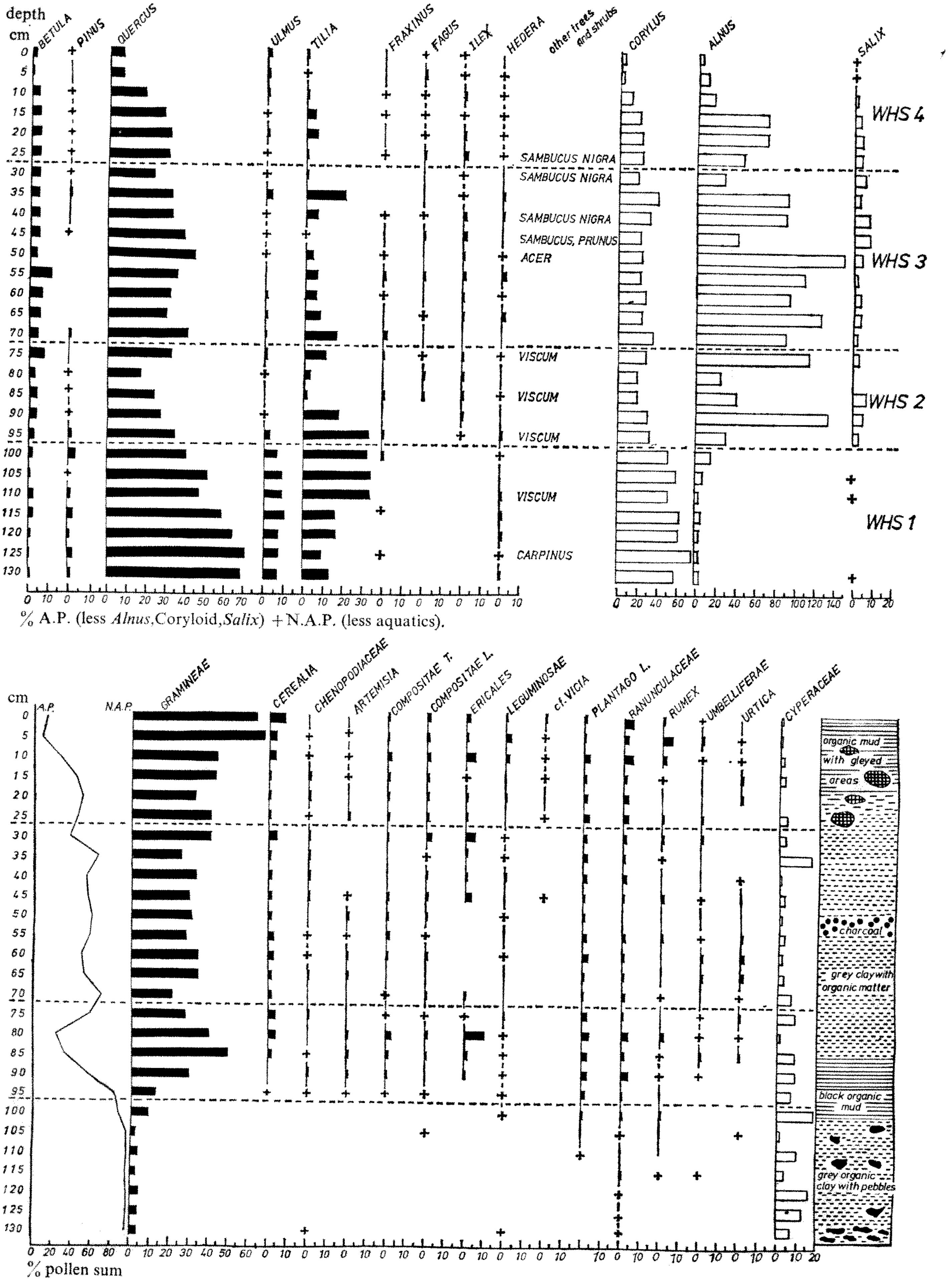

Fig. 1 Pollen diagram for West Heath Spa. Pollen counts of 400–900 grains were taken for each 5-cm layer.

counts of 400–900 pollen grains for each 5-cm layer. It can be divided into four zones of which 130–100 cm (WHS 1) corresponds to Zone VIIa, 100–75 cm (WHS 2) and 75–30 cm (WHS 3) correspond to VIIb and 30–0 cm (WHS 4) corresponds to VIII. These changes are reflected by the seed and beetle assemblages.

Quercus (oak) is the most commonly represented pollen type in WHS 1 but, *Tilia* (lime), an insect-pollinated tree, is believed to be under-represented in the pollen rain[1], and at West Heath Spa, its substantial pollen record seems to indicate a forest composed predominantly of lime. The abundance of lime pollen, seen also at Shustoke, Warwickshire[4], Butterbump, Lincoln (J.G., unpublished), and Epping Forest (P. A. Moxey and P. M. Oxford, personal communication) shows that contrary to earlier concepts of mixed oak forest during the Atlantic, lime was probably the dominant forest tree in the Midlands, southern and eastern England. The high values of oak and *Corylus* (hazel) compared with *Alnus* (alder) are probably due to the Spa's situation, an isolated marsh rather than a large wetland with surrounding alder carr. Amongst wood-dependent beetles, the commonest is *Ernoporus caucasicus* Lind., a small lime-feeder which has been found in numbers in deposits of this age[4], but was first captured in Britain as recently as 1954 (ref. 5). Other scolytids include *Kissophagus hederae* (Schmidt.), an ivy feeder, and the only host specific species in this country[6], and *Xyleborus xylegraphus* (Say) which is found on pines as well as several deciduous trees. Also present are the oak leaf-miner *Rhynchaenus quercus* (L.), the holly-feeding cerambycid *Pogonocherus hispidus* (L.) and the small anobiid *Gastrallus immarginatus* (Mull.)—a typical old forest species.

Delineating the boundary between Zone VIIa/VIIb, the pollen values for elm decrease dramatically and appearing for the first time are Cerealia and ruderals such as *Plantago lanceolata* L. (ribwort plantain), which are associated with the activities of man. Accompanying this classic elm decline are falls in the level of oak and lime and *Corylus* pollen. The appearance of *Fagus* (beech) in WHS 2 and maximum frequencies of *Ilex* (holly) in WHS 3 might indicate their expansion into areas of previously closed forest. The appearance of Ericales pollen denotes the start of heath formation. The insect fauna of WHS 2 contains a smaller percentage of tree obligate species than lower samples, although records of decaying wood inhabitants such as *Eremotes ater* (L.) are noteworthy, particularly if, as Rackham suggests, primitive man was unable to clear away large stumps and trunks of felled trees[7]. Both seeds and beetles reflect increased wetness leading to pond formation. Aquatic Ranunculaceae including *Ranunculus flammula* L. and *R.* (*Batrachium*) spp. are present together with *Isolepis setacea* (L.) R.Br. (bristle scirpus) and *Lycopus europaeus* L. (gypsywort). Seeds of *Pedicularis sylvatica* L. (lousewort) have been recorded for the first time in a Zone VIIb deposit. The beetle fauna is dominated by aquatic species of *Helophorus, Hydrochus* and *Hydraena.*

After the elm decline, a semi-stable state is maintained in WHS 3, perhaps by a succession of clearance episodes, the concentration of charcoal suggesting the use of fire. Renewed heath formation is inferred from the reappearance of Ericales. A new faunal element, dung beetles of the *Aphodius, Onthophagus* and *Geotrupes* genera, which appears at 80 cm, probably indicates grazing by herbivorous animals in the grasslands.

The final pollen zone, defined by a further steep fall in tree pollen, including *Corylus* and *Alnus,* with a corresponding rise in herbaceous pollen, is characteristic of pollen diagrams from the Iron age onwards. A similar pollen diagram has been drawn up from New Palace Yard, a London site dated to the Iron age (J.G. and S. Limbrey, unpublished). The continuing record of Gramineae, Cerealia, Leguminosae, *Plantago* and *Rumex* (dock) indicates further cultivation, and Ericales are present throughout. *Sirodendron cylindricum* (L.), is one of the very scarce wood-dependent beetles from these upper samples, most of the species require open land or waterside habitats, such as *Hydrothassa marginella* (L.) and *Plateumaris discolor* (Panz.), a cottongrass-feeder which favours acidic habitats.

Further investigation of all the organic remains at West Heath Spa should provide important evidence on several aspects of the prehistory of the area. It should be possible to determine closely the environment of the Mesolithic habitation site, to investigate man's increasing modification of the landscape after the elm decline and finally, to evaluate the anthropogenic factor in the formation of Hampstead Heath.

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Summer food as the factor limiting roe deer population size

THE sizes of unexploited populations of roe deer (*Capreolus capreolus*) are regulated by spring migration. Individuals which have been able to establish a territory (bucks) or home range (does) force all other animals to move out of the habitat. This phenomenon is specific to age class, and if there is no mortality amongst adult animals, most yearlings emigrate to find new forest area[13]. Thus, in order to develop an intensive harvest policy for roe deer population, knowledge of the number of animals which can be supported by the habitat (that is, social carrying capacity) is essential to avoid undesirable population losses through emigration. During 1970–75, an intensive team research study was carried out in various regions throughout Poland[6]. Deer food resources were estimated by the harvest plot method[3], and winter population densities were precisely determined by drive census techniques[11]. Based on these data, several study areas were selected in which hunting and natural mortality were very low, and where it was known that most juvenile animals emigrated each spring. We show here that summer food is a major factor limiting roe deer population size.

Census data included the proportion of fawns in the population. Amongst adults, I assumed a constant proportion of 2.01 females per male as determined by Strandgaard[13]. Adding the number of adult males and females killed by hunters, it was possible to determine the density of resident bucks (*RB*) and resident does (*RD*) in various forest types. These values were plotted next on log–log scale, against the summer food supply (*SF*), that is, deciduous browse and dicotyledonous ground flora production, because such components constitute basic summer diet of roe deer[12] (Fig. 1).

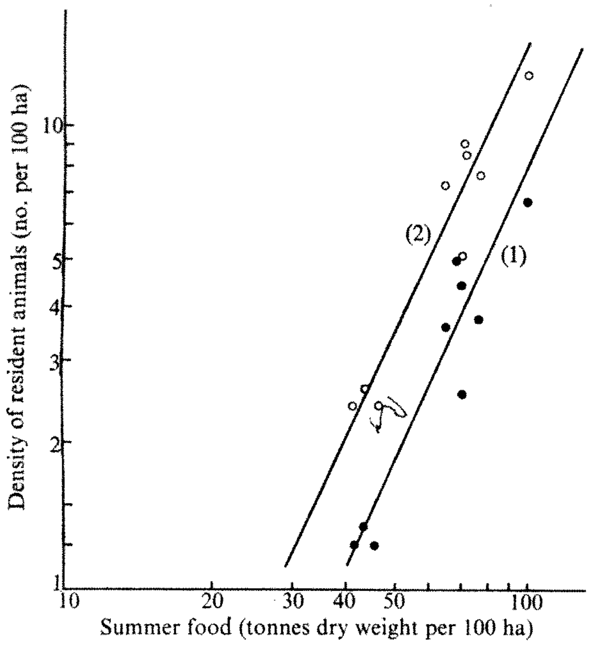

Fig. 1 Influence of summer food supply on the density of resident bucks and does. Based on studies of roe deer in Poland, 1970–75. Curve (1), $Y = 0.00058\ X^{2.06}$ (bucks). Curve (2) $Y = 0.00114\ X^{2.06}$ (does). $r = +0.93$.

Regression analysis was used to determine the relationship between the $\log_{10}$ of the density of bucks and the $\log_{10}$ of the total biomass of available summer forage (metric tons per 100 ha) for various localities. The correlation was very high ($R = 0.93$, $P < 0.05$) and is described by the formula

$$RB = 0.00058\ SF^{2.06} \tag{1}$$

Since the proportion of females is assumed above to be constant in the population, the correlation is also +0.93 and the equation determined by least square regression is

$$RD = 0.0014\ SF^{2.06} \tag{2}$$

These findings suggest that food resources are an important factor determining the number of resident bucks and does during summer season. This means that animals living in the forest with high availability of summer food, occupy smaller territory size than those inhabiting forest with poor summer food availability. Thus, territory size (TER) or home range (HR) area determines the number of resident animals per unit area; that is, the social carrying capacity of habitat, and this value may be defined by the equation

$$SC = \frac{1}{TER} + \frac{1}{HR} \tag{3}$$

This idea is important because in roe deer the summer availability determines territory or home range size, which then dictates the social carrying capacity of the habitat[13]. This is in contrast to the often held idea that the density of animals influence the territory size.

The territory size is also correlated with the age of roe deer. Using Strandgaard's data on the age of bucks and their territory size, the next relationship was determined[13]

$$Y = 11.02\ age^{0.616} \tag{4}$$

Using equation (1) and (2), and assuming the same exponent of the age of does in equation (4) to determine their home range, the territory or home range size may be expressed as function of both summer food supply and age of animal

$$TER\ (\text{ha}) = 72118.2\ age^{0.616}\ SF^{-2.06} \tag{5}$$

$$HR\ (\text{ha}) = 36632.6\ age^{0.616}\ SF^{-2.06} \tag{6}$$

Table 1 contains comparisons on summer and winter food resources for roe deer in seven common forest types in Europe[2]. Knowing total winter food consumption by a roe deer (56.3 kg dry weight; ref. 14), and using equations (3), (5) and (7), the summer (social) and winter (trophic) carrying capacities of these habitats were calculated.

If winter snow depth did not exceed 15 cm (ref. 2), the determining factor in roe deer population density is the summer carrying capacity, expressed by variation in territory size. Except the alder forest and possibly, the fresh deciduous forest type (fresh referring to mesic soil conditions), the winter carrying capacity has no effect on the density of roe deer populations. If one season reproduction results in a doubling of the population size, the winter forage can potentially limit the density in alder and fresh deciduous forest (see Table 1).

As a consequence of territoriality, roe deer possess a density-independent reproductive rate. There was a positive correlation between population density and the average number of corpora lutea per female (Table 2), but this correlation is not statistically

Table 2 Roe deer population density and average number of corpora lutea per adult female in different parts of Europe

Population density (Animals per 100 hectare) (X)	Corpora lutea per adult female (Y)	Country
13.0	2.00	Denmark (data from ref. 8)
13.3	1.85	Poland (ref. 4)
13.4	1.89	England (ref. 10)
14.9	2.18	England (ref. 10)
28.6	1.90	Poland*
56.0	2.40	Denmark (ref. 13)
63.0	1.90	Denmark (ref. 13)
63.0	2.00	Denmark (ref. 1)

$Y = 1.95 + 0.0022X$; $R = +0.27$, $P > 0.05$; corpora lutea maximum : minimum = 1.3.

* B.B., unpublished.

Table 1 Natural food resources of roe deer, their average territory and home range size, as well as summer and winter carrying capacity of some European forests

Forest ecosystems	Food-tons dry weight per 100 hectare Summer	Food-tons dry weight per 100 hectare Winter	Territory size of bucks (hectare)	Home range of does (hectare)	Carrying capacity of forest (no. per 100 hectare) Summer	Carrying capacity of forest (no. per 100 hectare) Winter
Fresh coniferous	40.4	13.9	69.6	35.4	4.3	246.9
Fresh mixed coniferous	83.3	8.6	15.7	8.0	18.9	152.7
Moist mixed coniferous	64.3	6.6	26.7	13.6	11.1	117.2
Mixed deciduous	70.4	3.4	22.2	11.3	13.3	60.4
Mountain deciduous	30.0	6.1	128.5	65.3	2.3	108.3
Fresh deciduous	72.9	1.5	20.6	10.5	14.4	26.6
Alder	112.4	2.0	8.5	4.3	32.5	35.5

The data are based on studies in Poland in 1970–75.

significant ($R = +0.27$, $P > 0.05$). It is well known that non-territorial populations of white-tailed deer (*Odocoileus virginianus*) and mule deer (*Odocoileus hemionus*) show a significant negative correlation between population density and reproductive rate. The average number of corpora lutea per female decreases as mule deer population increases ($R = -0.98$ (ref. 7)). Also, the same strong negative correlation was shown for white-tailed deer, regarding ova ($R = 0.92$) and embryos ($R = 0.90$) produced per female[7]. The ratio of maximum/minimum value of corpora lutea per female (a measure of variability) was recorded as 1.9 for mule deer[9] and 3.1 for white-tailed deer[5], but was only 1.3 in roe deer populations (Table 2).

Such small variability of number of corpora lutea per roe deer female, and the lack of correlation between density and reproductive rate probably result from spring social regulation of the population. As the territory is established to provide an individual with an adequate food resource, each individual of the same age possesses a territory large enough to provide equal amounts of summer food. Evidence which supports this thesis is the fact that the number of corpora lutea in a fenced roe deer population dropped drastically to 1.6, while free living roe deer in the same vicinity showed 2.1 per female[8].

Although this study was initiated to facilitate the intensive management of European roe deer populations, the results presented here have clear implications for an understanding of the natural regulation of populations of large herbivores.

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Injury requirement for initiation of regeneration of newt limbs which have whole skin grafts

THERE are three known requirements for salamander limb regeneration—injury, nerves, and wound epidermis[1,2]. During the past few years we have examined cellular events in amputated, regenerating limbs and compared these with non-regenerating limbs after either denervation[3,4] or after whole skin grafts over the amputation surface[5]. Results of these experiments led to the formulation of an hypothesis[6] which suggests unique roles for injury, nerves, and the wound epidermis during the initiation of regeneration: injury is important to cause dedifferentiation of limb stump tissue cells, entry of these cells into G_1 of the cell cycle and DNA replication; nerves are important for one or more G_2 events and thus indirectly for mitosis; and the wound epidermis maintains these cells in the cell cycle so that significant outgrowth (blastema formation) can occur. In the present study we have examined the extent and kinds of injury necessary to initiate regeneration in newt limbs which have had skin grafts for 5 weeks and externally show no regeneration. The results suggest that non-cycling dedifferentiated cells are present in 5-week skin graft limbs which immediately begin cycling when a wound epidermis forms after re-injury.

Whole skin was grafted over the amputation surfaces of 57 adult newt (*Notophthalmus viridescens*) limb stumps immediately after amputation of the limbs through the proximal portion of the radius and ulna as previously described[5]. Newts remained anaesthetised on moist gauze for 2 h and were positioned so that pressure from the gauze enhanced healing of the graft. Five weeks after amputation and skin graft, 44 of these limbs showed no external signs of regeneration. The other limbs either regenerated normally (six cases) or regenerated at a right angle from the area of skin flap overlap (seven cases) as seen in previous experiments.

These 44 non-regenerating limbs with skin flaps were either maintained as controls without re-injury or injured by: (1) a fresh amputation 1 mm proximal to the original amputation, (2) careful removal of the entire skin flap with watchmakers' forceps in order to expose the original amputation area in its entirety, and to allow new wound epidermis to form, (3) careful removal of a third of the original amputation surface to expose an area equal to only a third of the original amputation surface, (4) 15 penetrations through the skin flap with a sharp, sterile needle to a depth of 1 mm into the stump tissue, or (5) cutting a single transverse incision across the limb stump with a sterile razor blade to a depth of 1 mm into the stump tissue. We attempted to make these incisions parallel with the plane of the skin flap but they occasionally varied by as much as 20°.

The results of these various methods of injury can be seen in Table 1.

Table 1 Regeneration responses after re-injury of non-regenerating newt limbs containing skin grafts*

Type of re-injury	Number of skin graft limbs	Regeneration Yes	No
Control: no re-injury, skin graft remains intact	6	0	6
Re-amputate 1 mm into stump	8	8	0
Remove entire graft to expose entire amputation surface	8	7	1
Remove 1/3 of graft to expose 1/3 of amputation surface	5	4	1
15 needle penetrations 1 mm into stump	8	0	8
Transverse razor incision to a depth of 1 mm	9	8	1

*Limbs with skin grafts were examined 5 week after grafting. Only those which showed no regeneration were re-injured. Limbs were observed externally for regeneration at weekly intervals for 4 week.

Of the various methods used to re-injure skin graft limbs, all elicited regeneration responses with the exception of the 15 needle penetrations (Table 1). It is important to note that control amputations, removal of the entire skin graft, and removal of $\frac{1}{3}$ of the graft, all resulted in wound epidermis formation over the amputation surface, albeit only $\frac{1}{3}$ of the area in the latter case. When $\frac{1}{3}$ of the skin graft was removed, the wound area did not increase in size. Instead, regeneration occurred from the small wound surface and a small regenerate with apparently normal morphogenesis was the result.

It is surprising that 15 needle penetrations through the skin flap did not result in regeneration. Since the needle penetrations were to a depth of 1 mm into the stump, clearly some injury of stump mesodermal tissues occurred. The 15 individual wounds either did not retain free wound epidermis or the small wound epidermis areas were

Table 2 Enhanced regeneration of skin graft limbs re-injured by transverse incision compared with control amputated limbs*

Type of re-injury	Week of observation	Stages of regeneration reached Pre-mound	Mound	Cone	Paddle	Digit
Transverse incision	1	3	6	0	0	0
Amputation	1	9	0	0	0	0
Transverse incision	2	2	3	2	2	0
Amputation	2	9	0	0	0	0
Transverse incision	3	1	1	3	3	1
Amputation	3	4	5	0	0	0
Transverse incision	4	1	0	3	4	1
Amputation	4	0	6	3	0	0

*Only 5-week skin graft limbs which showed no external signs of regeneration were used for these re-injury experiments. The transverse incision was made to a depth of 1 mm into the stump. Skin grafts were made only on left limbs at which time right limbs were not amputated. Thus at 5 weeks, right limb controls were amputated for the first time. All control limbs and eight out of nine re-injured skin graft limbs eventually completed regeneration. Newts were maintained at 21 ± 1 °C throughout the experiment.

insufficient for regeneration to occur. A complete histological study of these re-injured limbs now in progress in our laboratory should clarify this problem.

Regeneration after re-injury of skin flap limbs could conceivably require dedifferentiation of stump tissues as well as wound epidermis formation[6]. According to the cell cycle hypothesis of Tassava and Mescher[6], the wound epidermis prevents re-differentiation of dedifferentiated stump tissue cells. In skin flap limbs, Mescher[5] found dedifferentiation, DNA synthesis, and mitosis 1 week after amputation to be equal to that of control amputated limbs with wound epidermis. However, by 2 weeks these parameters decreased significantly in skin flap limbs but increased in control limbs. Tassava and Mescher[6] and Mescher[5] suggested that in the absence of the wound epidermis, cells left the cell cycle and re-differentiated. The present results indicate that complete re-differentiation of limb stump cells does not occur in skin graft limbs, at least not during the 5 weeks following grafting. Particularly significant in this regard are the results of the transverse incision re-injury (Tables 1 and 2). In these limbs, a ridge formed along the incision within 4 d of re-injury in eight out of nine cases and mound blastemas were present by 1 week. From Table 2 it can be seen that some of these regenerates reached cone and even paddle stages of regeneration by 2 weeks after re-injury and digit stages by 3 weeks. Regenerates of control amputated limbs lagged far behind (Table 2). It is important to note that even at 26 °C, control newt limbs do not reach paddle or even cone stages by 2 weeks post-amputation[7]. In the present study, regeneration stages were also reached very early after entire or $\frac{1}{2}$ skin graft removals, but accurate staging data was not obtained.

What is likely, therefore, is that a significant number of dedifferentiated cells are present in skin flap limbs which are not traversing the cell cycle and thus are not seen as mitotic figures and do not label with ^{3}H-thymidine[5]. Thus when a wound epidermis forms after re-injury, these cells immediately begin cycling and dividing. Regeneration of control limbs is therefore slower because of the time required for dedifferentiation. Our preliminary histological observations are consistent with this view. It should now be possible to determine which stage of the cell cycle these dedifferentiated cells are in and whether, after even longer post-graft times, that is 8–10 weeks, similar re-injuries will elicit regeneration. These latter experiments are particularly important since Polezhaev[8] observed no regeneration when whole skin grafts were removed from axolotl limb stumps after an 8–9 week graft period. Re-injury of newt limbs with 10-week-old skin grafts will test whether dedifferentiated cells are still present and capable of blastema formation.

The regenerates which developed after a transverse incision is made through the skin flap always formed in the plane of the incision. Since some of these incisions varied by as much as 20° from the normal dorsal ventral axis of the limb, the wound epidermis seems to have a strong directional influence on regeneration, consistent with Thornton's[7] early findings. Additional experiments further varying the axis of the incision, and removing varying areas (and shapes) of skin grafts, will provide additional insights into the role of the wound epidermis in regeneration.

The results of these experiments confirm Mescher's[5] results that cell dedifferentiation initially occurs in skin graft limbs in the complete absence of a wound epidermis but DNA synthesis and mitosis then subside without outgrowth. These cells apparently remain dedifferentiated for 5 weeks since rapid blastema development is seen after re-injury and wound epidermis formation. These findings are consistent with the views presented by Tassava and Mescher[6] that the wound epidermis is not required for dedifferentiation but that it is important to keep dedifferentiated cells moving through the cell cycle.

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Bilateral interaction in olfaction

THERE has been much interest in perceptual interactions between the ears and between the eyes, but much less in those between the nostrils, or more precisely, the two sides of the olfactory system[1–4]. This report reveals that, for a wide range of supraliminal stimulation, there is considerable interaction between the two sides. The facets considered here are bilateral additivity of odour intensity, and bilateral influence of an adapting stimulus presented unilaterally.

In the experiment on bilateral additivity, 20 subjects estimated the intensity of various concentrations of *n*-butyl alcohol presented to either or both nostrils. There were six stimuli—five concentrations of odorant prepared in aqueous solution plus plain water. Samples (1 ml) were placed in small vessels designed for olfactory testing and for ready assessment of vapour phase concentration[3]. Concentrations of odorant, assessed chromatographically, varied from 0.10 to 7.60 mg l^{-1} of air in a geometrical progression. On each trial, the subject sniffed the contents of two vessels simultaneously, one placed at the right and the other at the left nostril. On the first trial, both vessels contained 0.86 mg l^{-1}. The subject was instructed to assign the number 10 to the odour magnitude produced on this trial. On subsequent trials, the subject judged various left-right and right-left combinations formed from the six concentrations, including water. Of the 36 possible combinations only one, water–water, was excluded. The subject assigned numbers proportional to odour magnitude. Thus if a particular combination smelled three times as strong as the first, the subject called it 30, and so on (method of magnitude estimation)[6]. Order of presentation was irregular. Each subject served in two sessions, identical except for order of presentation.

The results, averaged geometrically across subjects, yielded a family of psychophysical functions relating odour intensity to concentration. Isointensity contours constructed from this family offer a way to decide on the degree of bilateral interaction and to compare the results reported here with analogous results in other modalities (Fig. 1)[2,7]. The contours show the combinations of concentrations to the right and left nostrils that yielded each of several odour intensities, $\psi = 5$ to $\psi = 35$.

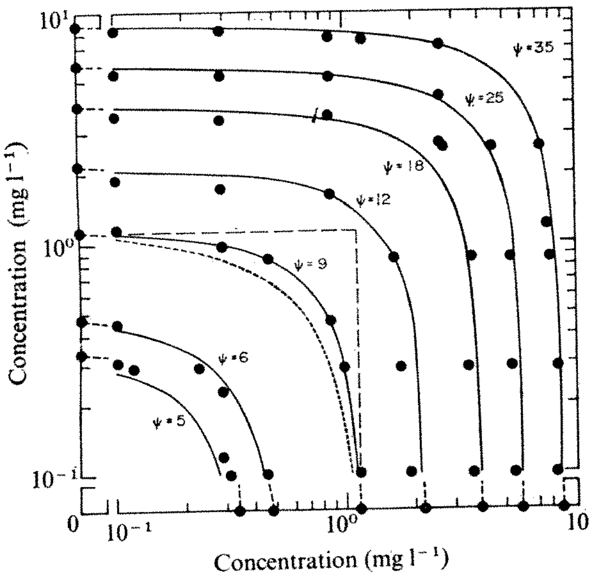

Fig. 1 Data points show the various combinations of concentrations in the right and left nostrils that led to equal perceived intensity (ψ) at various levels ranging from 5 to 35. The dashed rectilinear contour exhibits the shape that would have resulted if the perceived magnitude of a bilateral complex had equalled the magnitude of the higher concentration of the complex smelled alone. The dotted contour exhibits the shape that would have resulted if the perceived magnitude of the complex were governed by the total amount of stimulus, irrespective of how it was distributed between the nostrils. The solid contours depict the vector sum of the mass of odorant in the right and left nostrils (see equation (1)). The angle between the vectors was 60°.

The shapes of the contours showed that olfaction behaves neither like audition, where the loudness of a binaural complex falls close to the sum of the loudnesses of the monoaural components, nor like vision, where the brightness of a binocular complex characteristically falls below the brightness of the stronger monocular component[8,9]. Other straightforward possibilities merited consideration. If the perceived magnitude of the odour complex had equalled the magnitude of the higher concentration of the complex smelled alone, the contours would have a rectilinear shape (see dashed contour in Fig. 1). Alternatively, if the perceived magnitude of the complex had been governed by the total amount of stimulus, irrespective of how it was distributed between the nostrils, the contours would have the shape shown by the dotted contour. The outcome fell between these two possibilities, but resembled more closely the dotted contour, which portrays stimulus summation.

Perfect stimulus summation can be described by the equation

$$\varphi_{ab}=(\varphi_a^2+\varphi_b^2+2K\varphi_a\varphi_b)^{1/2} \qquad (1)$$

where φ_{ab} is the mass of odorant in the complex, φ_a and φ_b are the masses of the components, and where K equals 1.0. Values of K less than 1.0 reflect degrees of partial summation. To decide whether partial summation could account for the degree of bilateral additivity, the data in Fig. 1 were used to solve for K. The value obtained, 0.5, gave rise to the solid contours. These offer a good account of the results. Accordingly, the olfactory system behaved as if the effective magnitude of the stimulus in a bilateral complex were the resultant of the vector sum of the magnitudes in the components. A K of 0.5 implies a 60° angle between the vectors.

The rule of partial summation exhibited here suggests that when there is an imbalance of stimulation to the nostrils, the side receiving the stronger stimulus makes a disproportionate contribution to total intensity. This is seen in the shapes of the isointensity contours. When the magnitude of stimulation is strong on one side and weak on the other, a gradual increase in the weaker stimulus requires at first only a small compensatory decrease in the stronger in order to maintain perceived intensity constant. As the weaker stimulus approaches the magnitude of the stronger, however, the compensatory decrease becomes considerably larger, and indeed very large when the balance of stimulation shifts from one side to the other. Such behaviour could reflect inhibitory interaction between the olfactory bulbs, a process already seen electrophysiologically and one invoked to account for why the course of adaptation in rats is slowed when the anterior commissure between the bulbs is transected[10–12].

Evidence for bilateral interaction in olfactory adaptation has come also from studies of thresholds in human subjects[13–15]. Specifically, an adapting stimulus presented unilaterally serves to raise the absolute threshold for both ipsilateral and contralateral nostrils. But, the ipsilateral side shows greater and longer lasting loss of sensitivity than the contralateral side. These results implicate bulbar, or other central neural structures, in the process of adaptation[16]. It has yet to be shown, however, that the ability of an adapting stimulus to transfer its influence contralaterally occurs on the supraliminal level and with a brief monorhinic adapting stimulus that eliminates the possibility of retronasal leakage of odorant to the contralateral side. To check these possibilities, 30 subjects judged the intensity (magnitude estimation) of various concentrations of linalyl acetate presented after an adapting stimulus to the nostril ipsilateral or contralateral to the test nostril. On each trial, the subject sampled the contents of two vessels successively, but during the same continuous inhalation (4 s duration). Both adapting and test stimuli were presented on the same inhalation in order to eliminate any backflush of odorant to the contralateral posterior naris. Both nostrils were always open, thereby insuring a continuous flow of air from the anterior to the posterior nares throughout inhalation. The first vessel presented on a trial could contain just the diluent (diethyl phthalate) or linalyl acetate (0.53 mg l^{-1}, the adapting stimulus). The second could contain any of five test concentrations (0.02–1.60 mg l^{-1}). The first vessel could be presented to either the right or the left nostril, and the second could be presented ipsilaterally or contralaterally with respect to the first. The various combinations of right–left, ipsilateral–contralateral, diluent-test stimulus, adapting stimulus-test stimulus were intermingled randomly within a session. The subjects waited approximately 1 min between trials.

Figure 2 shows the psychophysical functions obtained (1)

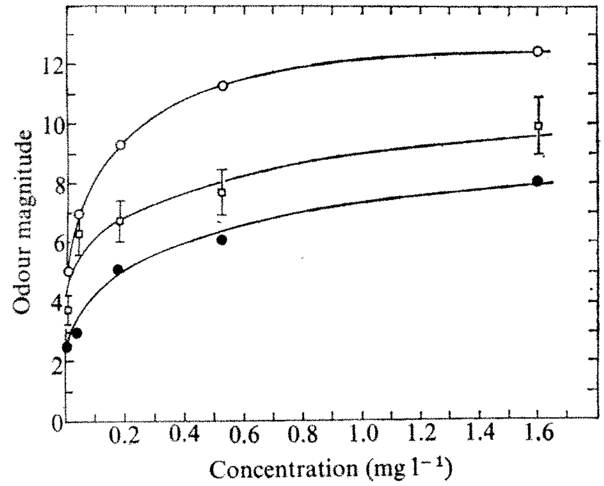

Fig. 2 Psychophysical functions for linalyl acetate obtained when test concentrations succeeded the diluent (no adaptation, ○) and when they succeeded an adapting stimulus (0.53 mg l^{-1}) on the same side (ipsilateral adaptation, ●) or on the opposite side (contralateral adaptation, □). The vertical bars represent the standard error of the difference between the results for no adaptation and contralateral adaptation (upper bars) and between the results for contralateral adaptation and ipsilateral adaptation (lower bars).

when the test stimuli succeeded the diluent, (2) when they succeeded linalyl acetate (0.53 mg l^{-1}) contralaterally and (3) when they succeeded the same concentration of linalyl acetate ipsilaterally. The functions line up in a manner consistent with bilateral interaction. Ipsilateral adaptation was characterised by a marked decrease in perceived magnitude throughout the entire range of concentrations; contralateral adaptation was characterised by a somewhat less marked, but still substantial, decrease. The difference between the two adaptation curves reflects in part the degree of peripheral neural involvement in the adaptation process. But the difference presumably reflects also any attenuation in the effectiveness of the adapting stimulus as its influence transfers contralaterally at some point in the central nervous system.

In summary, there is considerable bilateral interaction in the olfactory system. The interaction is revealed in bilateral additivity of intensity and in adaptation. The interaction seems to be independent of level of stimulation, for the same equation, based on vector addition, can describe the degree of bilateral additivity at both high and low levels of perceived intensity. The equation implies partial summation of stimulus magnitude and suggests that the nature and degree of bilateral interaction in olfaction is unique among the senses.

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Bicyclic phosphorus esters increase the cyclic GMP level in rat cerebellum

4-ALKYLDERIVATIVES of 1-phospha-2,6,7-trioxabicyclo[2.2.2] octane-1-oxide (PTBO derivatives) are highly toxic organophosphates. On intraperitoneal injection to mammals they produce tonic–clonic convulsions and death within a few minutes[1,2]. Their mechanism of action is unknown, but it is different from that of other toxic phosphorus esters which act as inhibitors of cholinesterase[1]. The symptoms of poisoning by PTBO derivatives indicate an activation of the central nervous system[2]. There is evidence suggesting that the cyclic nucleotides AMP and GMP act as second messengers in central and peripheral synapses and so we have investigated the effects of PTBO compounds on the level of these nucleotides in the brain. The ethyl (EPTBO) and isopropyl (IPTBO) compounds, two of the most toxic PTBO derivatives, were used. The results show that following intraperitoneal injection of these compounds into rats, the cyclic GMP concentration is increased in cerebellum by both convulsive and subconvulsive doses, whereas the levels of the nucleotide are unchanged in cerebral cortex and subcortical tissues. The increase in cyclic GMP level in the cerebellum is thus not due to the convulsive state.

The PTBO derivatives were synthesised at FOA 4 according to the method of Wadsworth and Emmons[3]. The LD_{50} value (intraperitoneal, rats) of EPTBO was 1.1 mg kg^{-1} and that of IPTBO 0.1 mg kg^{-1} which is in agreement with values reported by others[2]. Atropine sulphate 20 mg kg^{-1} injected intraperitoneally 20 min before EPTBO did not change the toxicity of the convulsant. Diazepam 5 mg kg^{-1} administered similarly 10 min before EPTBO increased the LD_{50} value to 2.5 mg kg^{-1}.

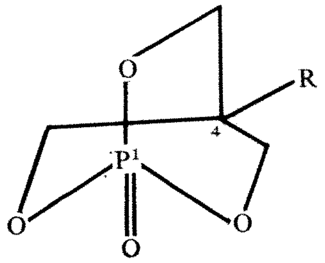

Fig. 1 4-Alkyl-1-phospha-2,6,7-trioxabicyclo[2.2.2]octane-1-oxide. EPTBO: R = ethyl; IPTBO: R = isopropyl.

Cyclic nucleotides were assayed in the brains of Wistar male rats (160–300 g) injected intraperitoneally with PTBO compounds and of controls injected with 0.9% NaCl. The rats were killed by microwave irradiation (2,000 W) for 10 or 40 s for cyclic AMP and cyclic GMP respectively. Animals injected with convulsive doses (EPTBO, 1.2 mg kg^{-1}) were killed during the third tonic convulsion (about 15 min after injection). Control animals and rats given subconvulsive doses (IPTBO, 0.05 mg kg^{-1}) were killed after the same time. In some experiments diazepam (5 mg kg^{-1}) or atropine sulphate (20 or 50 mg kg^{-1}) were injected intraperitoneally before PTBO. Tissue pieces from cerebral cortex, subcortical tissues and cerebellum were homogenised at 4 °C and extracted according to Folbergrova[4]. Cyclic AMP and cyclic GMP were assayed using test kits (Radiochemical Centre, Amersham, England).

Table 1 Effects of EPTBO on the levels of cyclic AMP and cyclic GMP in different brain areas

	Cyclic AMP pmol per mg wet weight			Cyclic GMP pmol per mg wet weight		
	Control	During convulsions	*P*	Control	During convulsions	*P*
Cerebral cortex	0.32±0.05 (17)	0.34±0.11 (13)	n.s.	0.089±0.013 (6)	0.105±0.014 (6)	n.s.
Subcortical tissues	0.40±0.08 (15)	0.45±0.15 (11)	n.s.	0.137±0.013 (5)	0.187±0.032 (6)	n.s.
Cerebellum	1.29±0.21 (21)	1.74±0.27 (15)	n.s.	0.593±0.054 (8)	2.708±0.338 (8)	<0.001

Values represent the mean±s.e.m. for the number of animals given in parentheses.
P values were obtained by *t*-test; n.s. not significant.

Convulsive doses of EPTBO caused no significant differences in the levels of cyclic AMP in any of the brain areas studied compared with control animals (Table 1). The level of cyclic GMP was, however, increased almost fivefold (470% of the control values) in the cerebellum of rats with PTBO-induced convulsions whereas it was unaffected in the other brain areas studied (Table 1). A subconvulsive dose of IPTBO increased the level of cyclic GMP in cerebellum almost twofold (167% of the control values) (Table 2). Pretreatment with atropine sulphate did not significantly alter the effect of the PTBO derivatives on the cyclic GMP level. Diazepam given 10 min before the administration of the PTBO derivatives, however, blocked the increase of cyclic GMP induced by the subconvulsive dose of IPTBO and reduced the increase by the convulsive dose of EPTBO from fivefold to less than twofold (Table 2).

The elevation of cyclic GMP in the cerebellum seems not to be due to the convulsive state of the animals since a subconvulsive PTBO dose also caused an increase. The cyclic nucleotide content of postsynaptic cells in neural tissues is regulated by transmitters released from nerve terminals impinging on the cells[5,6]. Increase of cyclic GMP in the brain might be correlated to stimulation of the muscarinic cholinergic receptor (mAChR), but atropine, a well known mAChR blocker did not reduce the IPTBO-induced increase of cyclic GMP level in the cerebellum. In addition, atropine neither affected the convulsions nor decreased the toxicity of the PTBO compounds and preliminary results showed no effect of PTBO derivatives on acetylcholine release from cerebral cortex. This seems to rule out interference with muscarinic cholinergic neurotransmission.

The symptoms of PTBO poisoning are similar to those produced by bicuculline and picrotoxin, known blockers of γ-aminobutyric acid (GABA) receptors. Bowery *et al.*[7] have reported that PTBO compounds act as specific antagonists to the action of GABA. In addition we have found a slightly reduced K$^+$-induced release of ^{3}H-GABA from synaptosomes in the presence of PTBO derivatives[8]. GABA is the inhibitory transmitter released at many synapses in the cerebellum[9]. The PTBO-induced increase of cyclic GMP in the cerebellum might be caused by primary action of these agents on the inhibitory GABA mechanism. In support of this is the fact that the effects of the PTBO derivatives on the cyclic nucleotides—an increased cyclic GMP level in cerebellum and unaffected cyclic AMP—are in accordance with those described for some agents interfering with the GABA-ergic mechanism[10,11] and also that diazepam, suggested to exert a facilitory effect on GABA transmission[12], decreased both the PTBO-induced elevation of cyclic GMP and the toxicity of these compounds.

Table 2 Effects of atropine and diazepam on the PTBO-induced increase of cyclic GMP in cerebellum

Treatment	Cyclic GMP (pmol per mg wet weight)
Saline	0.593±0.054 (8)
IPTBO 0.05 mg kg^{-1}	0.989±0.048 (6)
Atropine 50 mg kg^{-1} + IPTBO	0.963 (2)
Atropine 20 mg kg^{-1} + IPTBO	0.958 (2)
Diazepam 5 mg kg^{-1} + IPTBO	0.576±0.084 (4)
EPTBO 1.2 mg kg^{-1}	2.708±0.338 (8)
Diazepam 5 mg kg^{-1} + EPTBO	1.048±0.082 (4)

Atropine 20 mg kg^{-1} and 50 mg kg^{-1} given intraperitoneally 15 and 30 min respectively before injection of IPTBO (0.05 mg kg^{-1}). Diazepam (5 mg kg^{-1}) was similarly injected 10 min before IPTBO (0.05 mg kg^{-1}) or EPTBO (1.2 mg kg^{-1}). Controls received 0.9% NaCl. Values are means ± s.e.m. for the number of animals given in parentheses.

The Purkinje cell axons of cerebellum participate in the regulation of motor activity initiated in cerebral cortex. Cyclic GMP is involved in the regulation of Purkinje cell activity and elevation of cyclic GMP in the cerebellum has been suggested to occur in those cells[9]. The cyclic GMP concentrations are suggested to be regulated by the interaction of excitatory and inhibitory transmitters[9]. Blocking of GABA action would increase the release of excitatory transmitters. The putative cerebellar transmitters glutamate and noradrenaline have both been reported to stimulate cyclic GMP synthesis in cerebellum[9,13]. The increase of cyclic GMP induced by the PTBO derivatives may be an expression of an increased release of excitatory transmitters from neurones synapsing on the Purkinje cells.

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A new class of GABA agonist

COMPOUNDS which mimic the activity of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) on postsynaptic receptors are of particular pharmacological interest as possible therapeutic agents in human neurological disorders and as molecular probes with which to study different types of GABA receptors. The GABA molecule has considerable flexibility with free rotation around all three carbon–carbon bonds as indicated in Fig. 1. This conformational mobility is reduced in GABA analogues such as trans-4-aminocrotonic acid[1] and muscimol[2], which are potent GABA agonists at bicuculline-sensitive receptors on spinal neurones of the cat. The conformational mobility of such compounds can be reduced still further by incorporating the amino function in a ring structure. This has led to a new class of GABA agonist described here, based on 1,2,3,6-tetrahydropyridine-4-carboxylic acid (isoguvacine) and the related bicyclic isoxazole 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP) (Fig. 1). Isoguvacine represents a semi-rigid analogue of trans-4-aminocrotonic acid in a folded conformation. THIP, a folded analogue of muscimol, is even more rigid as the 'masked' carboxyl group (the 3-isoxazolo moiety) is fixed in the general plane of the molecule. The action of these compounds as GABA agonists provides indirect evidence that GABA interacts with bicuculline-sensitive postsynaptic receptors in the cat spinal cord in a partially extended and almost planar conformation.

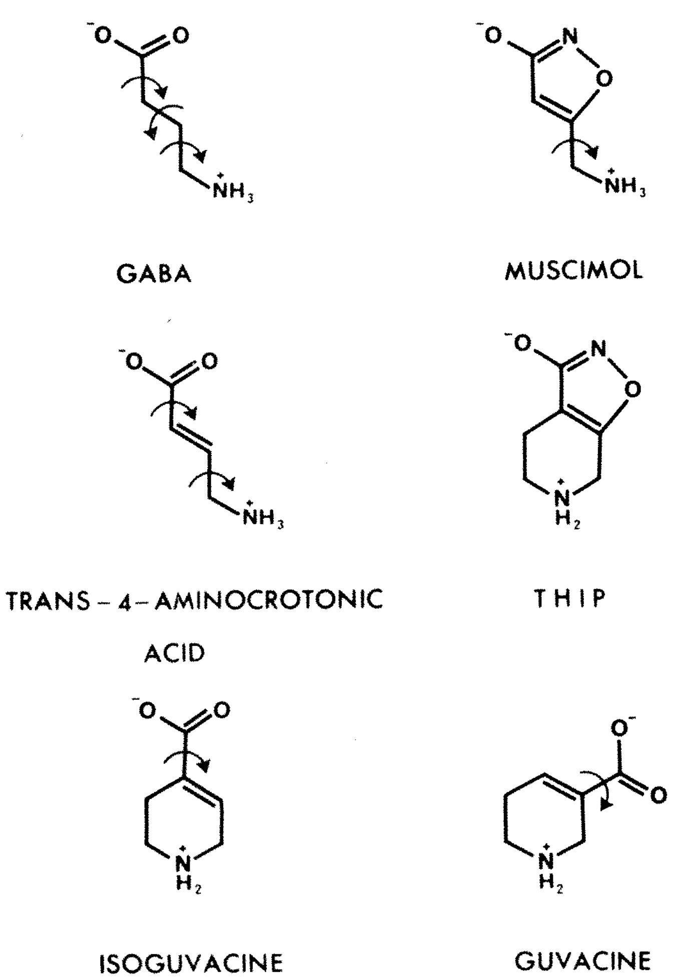

Fig 1 Conformational mobility of GABA and related compounds as illustrated by the rotational freedom around carbon–carbon single bonds marked with arrows.

Experiments were performed on lumbar dorsal horn interneurones and Renshaw cells of three cats anaesthetised with pentobarbitone sodium (35 mg per kg body weight intraperitoneally, supplemented when required). Extracellular action potentials were recorded by means of the centre barrel of seven barrel micropipettes and the compounds tested were administered electrophoretically from the outer barrels of the micropipettes[2], which contained 100 or 200 mM aqueous solutions adjusted to *p*H 3 except for bicuculline methochloride (BMC, 10 mM in 165 mM sodium chloride) and DL-homocysteate (DLH, 200 mM, *p*H 7.5). The approximate potency of any depressant action was assessed relative to that of GABA on the basis of electrophoretic currents required to produce equal and submaximal inhibitions of firing induced by DLH. *In vitro* experiments were carried out to determine the uptake of GABA (10 nM) by small slices (about 0.1 × 0.1 × 2 mm) of rat cerebral cortex[3], and the transamination of GABA catalysed by extracts of rat brain mitochondria[4]. The syntheses of the new compounds isoguvacine and THIP will be described elsewhere. Muscimol and BMC were prepared by published procedures[5,6]. All other compounds were purchased from commercial suppliers.

The depressant action of isoguvacine (tested on 10 neurones) was comparable with that previously reported for muscimol[2], and was two to four times more potent than that of THIP (eight neurones) and GABA which were approximately equipotent. The depressant action of all of these compounds was reversibly antagonised by bicuculline methochloride in conditions where the inhibitory action of glycine was unaffected (Fig. 2). The saturated analogue of isoguvacine, piperidine-4-carboxylic acid, also depressed the firing of spinal interneurones (3) with a potency similar to that of GABA, and this action was also reversibly antagonised by BMC.

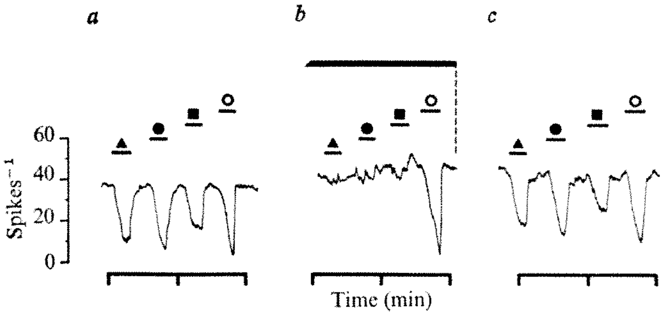

Fig 2 The effect of bicuculline methochloride (BMC) on the inhibition of the firing of a spinal interneurone by isoguvacine (35 nA; ●), THIP (60 nA; ▲), GABA (60 nA; ■), and glycine (15 nA; ○) ejected for the times indicated by the symbols and horizontal bars. The firing of the neurones was maintained with continuously ejected DL-homocysteate (DLH, 10 nA). The relative effectiveness of the inhibitory amino acids is similar to that observed in all experiments. *a*, Before; *b*, during BMC (15 nA) which was terminated at the vertical broken line; *c*, 3 min after (*b*).

As with the development of inhibitors of the neuronal uptake of GABA such as nipecotic acid[7] and guvacine[8], the present investigation arose from studies of the structure–activity relationships of isoxazoles related to muscimol. Isoguvacine and THIP were inactive at 0.5 mM as inhibitors of the neuronal uptake of GABA by slices of rat cerebral cortex, and also at 1 mM as inhibitors of the transamination of GABA catalysed by extracts of rat brain mitochondria. It is of interest to contrast isoguvacine and guvacine (Fig. 1) with respect to bicuculline-sensitive GABA receptors on spinal neurones and to transport carriers mediating the neuronal uptake of GABA in brain slices: isoguvacine is apparently a potent agonist with respect to these receptors and inactive against the carriers, whereas guvacine seems to be inactive as a GABA agonist[9] and a potent substrate-competitive inhibitor of the carriers[8]. These results are in agreement with the proposal that these particular postsynaptic receptors and transport carriers for GABA exhibit different structural specificities[10]: isoguvacine and guvacine may represent two different 'active conformations' of GABA selectively interacting with the bicuculline-sensitive postsynaptic receptors and neuronal uptake carriers respectively.

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Abnormal adenine metabolism of erythrocytes in Duchenne and myotonic muscular dystrophy

Duchenne muscular dystrophy (DUD) and myotonic muscular dystrophy (MYD) are inherited as sex-linked recessive and autosomal dominant traits, respectively. Although the primary manifestation is progressive muscle weakness, multiple organ systems seem to be affected. Cardiac abnormalities and mental retardation are described in both conditions, and defects in immunoglobulins and endocrine functions have been reported in MYD[1]. The primary inherited metabolic defect is unknown, but there is evidence to suggest an abnormal sarcolemmal membrane[1]. The possibility that muscle disease, especially involving membrane dysfunction, is reflected in the erythrocytes is supported by the demonstration of defective phosphorylation in the erythrocytes of patients with DUD[2] and MYD[3]. In DUD the erythrocytes appear abnormal in the scanning electron microscope[4] and in MYD electron spin resonance reveals abnormal membrane fluidity. There are other reasons to study erythrocytes, for the direct study of muscle is hampered by the need for repeated biopsies and by atrophy and fibrous tissue, which can cause secondary biochemical changes. Furthermore, the yield of isolated sarcolemmal membranes is often small and impure. In DUD, there is a 30% reduction in muscle ATP[6] and an abnormal ATP : ADP ratio of 1.57. Whether this is a significant defect or is a result of secondary changes in the muscle has yet to be clarified. We report here that the intracellular phosphorylation of adenine nucleotides derived from adenine incorporated by the erythrocytes is abnormal in both DUD and MYD.

Ten DUD patients from 10 families, five DUD carriers (two obligate, two probable, one possible) from five families, and seven MYD patients from five families were used and compared with 16 normal subjects, eight patients with other neuromuscular diseases, and four patients with a variety of disorders. 2.5 ml of heparinised venous blood was obtained from each patient in double blind conditions and spun at slow speed (800 r.p.m. 12-inch diameter rotor) for 17 min at 20 °C. The platelet-rich-supernatant plasma (PRP) was removed and separately centrifuged at 3,000 r.p.m. for 5 min to obtain platelet-poor-plasma (PPP). The red cell fraction after removal of PRP was centrifuged at 3,000 for 5 min to pack down the cells. 0.2 ml of packed red cells drawn from the centre of the sample was mixed with 0.2 ml of their own PPP in a polyethylene tube at 37 °C in a water bath. 10 µl of U-^{14}C-adenine (1 µCi) was added and incubation continued with gentle shaking for 30 min. The tubes were cooled briefly in ice and 0.5 ml of cold 13% perchloric acid (PCA) was added. After mixing and centrifuging at 3,000 r.p.m. the clear PCA supernatant (15 µl) was chromatographed on thin-layer cellulose sheets using a solvent of formic acid–teramyl alcohol–water (10 : 15 : 5). After drying each run was cut into 1-cm strips and the radioactivity determined on a liquid scintillation spectrometer[7]. Each peak was calculated as a percentage of the total ^{14}C-nucleotide pool formed during the incubation period and the profile of peaks run was summarised as a ratio R=(ATP+ADP)/AMP which describes the relative proportions of high-and-low energy compounds. All assays were performed within 2 h on fresh erythrocytes without freezing or storage.

The results on red cells suspended in their own plasma showed significant difference in the ratio of ATP+ADP to AMP of the patients, when compared with normals and disease control (Table 1). The mean ratio (R) for the normal subjects and disease controls were 9.6±4.1 and 8.7±4.6. MYD patients showed the greatest reduction in their ratio, with a mean of 2.9±1.8 but there was also a significant reduction in DUD (4.9±2.9). Thin-layer chromotography usually demonstrated a reduction in the proportions of both ATP ADP and an increase in AMP production. ^{14}C-adenine was present in all cases in excess so that it was not rate limiting, but the data suggested that adenine uptake was reduced in the DUD and MYD patients.

ATP exerts a protective effect on cell membrane integrity[8]. Thus any condition which leads to a decreased proportion of intracellular ATP is likely to result in leakage of enzymes and other proteins. As well as acting as a reserve of energy, ATP may have a direct effect in maintaining the integrity of the cell membrane, since it is concerned in the biosynthesis of phosphatidic acids, intermediates in the synthesis of phospholipids in membranes[9].

Our findings are indicative of appreciable metabolic disturbances in red blood cells of MyD and DuD, which are apparently insufficient to produce gross physiological

Table 1 Mean per cent of ^{14}C-adenine nucleotide pool in RBCs

	No. of patients	ATP	ADP	AMP	$R = \dfrac{(\text{ATP}+\text{ADP})}{\text{AMP}}$	P*
Normal controls (NC)	17	66±13	14±6.1	10±4.0	9.4±4.1	—
Disease conrols (DC)	12	66±8.2	16±8.1	14±10	8.7±4.6	NS
Duchenne (DUD)	10	46±19	22±5.6	19±12	4.9±2.9	0.005
Myotonic (MYD)	7	46±14	21±5.4	29±11	2.9±1.8	0.005
Duchenne carrier (DUD C)	5	57±22	8±1.6	22±23	5.1±2.6	NS

NS, not significant. Data are given ±s.d.
*P determined by Student's t test.

dysfunction and therefore may be a useful partial model for the further study of these genetic disorders.

The concept of failure to maintain a normal profile of adenine nucleotides may be an important factor in the membrane defect in Duchenne and myotonic dystrophy and needs further investigation.

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Amino acid sequence similarities between amyloid P component C1t and CRP

THE P component is a normal 9.5 S serum α1 glycoprotein composed of subunits that is found as a minor constituent of all types of amyloid deposits[1,2]. Painter *et al.*[3] have described a 9.5 S α1 glycoprotein, C1t, composed of ten 23,000 molecular weight (MW) subunits, which may have an important role in the regulation of complement C1 function. Ultrastructural and immunological studies showed a striking resemblance between P and C1t (refs 3, 4). CRP is an acute phase reactant, present in many species which can interact with pneumococcal C-polysaccharide, choline phosphatides and various polycations to activate the complement cascade[5,6]. It is composed of a 23,000 MW subunit non-covalently linked as a pentamer[7]. A clear-cut structural relationship between C1t and CRP has been demonstrated by comparing amino terminal sequences[7]. Since all three molecules—P, C1t and CRP—have a similar ultrastructure, namely, a cyclic pentamer or a decameric double, the term 'pentraxins' has been proposed by Osmand *et al.* for this group of molecules[7]. We report here the characterisation and the amino terminal sequence of human P component. The P component is identical to C1t except for the absence of the first residue (His), thus providing additional evidence for a close evolutionary relationship for P, C1t and CRP.

P component was isolated by saline extraction, Sephadex, diethylaminoethyl and cellulose chromatography, according to the method of Skinner *et al.*[2], from the liver of a patient (WIL) with secondary amyloidosis. By sodium dodecylsulphate–polyacrylyamide gel electrophoresis, the material consisted of a single 23,000 MW component which had a tendency to aggregate in reducing conditions. Figure 1 compares the amino acid sequence of the first 27 residues with those of C1t (ref. 7), three partially sequenced P components[2,4], and CRP (ref. 7). When compared to C1t, our preparation is one residue shorter at the amino terminal. The amino terminal residue could not be identified but the remaining 25 residues were identical. Position 19 is tentative since tyrosine was identified by thin layer chromatography and histidine by amino acid analysis after back hydrolysis. Differences between our P component and those reported previously[2,4] may be due to individual differences between different preparations or perhaps, in several instances, due to carry-over or other technical problems in residues that are difficult to identify. A closer similarity between C1t and P than between these molecules and CRP is suggested by the finding that both anti-P and anti-C1t antibodies gave a reaction of identity with P, as shown previously[4], while there was no antigenic relationship between P and CRP. Our anti-P antibody did not react with various preparations of CRP and four antibodies to native and denatured CRP did not react with P (WIL).

These results raise the possibility that there is a series of structurally closely related proteins which may have an important role in regulating and modulating certain immunological and inflammatory reactions[7]. Interestingly, our tissue-derived P component seems to lack the first residue, perhaps as a result of proteolytic cleavage. The structural and antigenic identity observed between C1t and P suggests a closer relationship between them than with CRP. But we do not yet have sufficient data to conclude that they are indentical. Study of the interaction of P with antigen antibody complexes, complement components

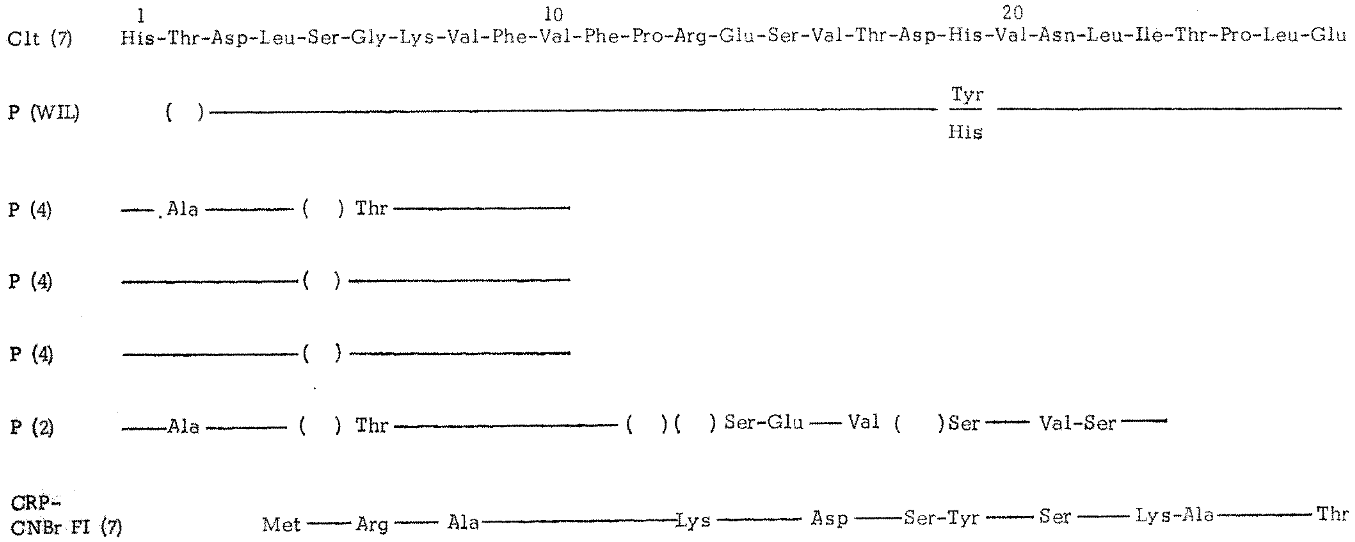
1
10
20
C1t (7)
His-Thr-Asp-Leu-Ser-Gly-Lys-Val-Phe-Val-Phe-Pro-Arg-Glu-Ser-Val-Thr-Asp-His-Val-Asn-Leu-Ile-Thr-Pro-Leu-Glu
P (WIL)
( )
Tyr
His
P (4)
.Ala ( ) Thr
P (4)
( )
P (4)
( )
P (2)
Ala ( ) Thr ( )( ) Ser-Glu Val ( ) Ser Val-Ser
CRP-
CNBr FI (7)
Met Arg Ala Lys Asp Ser-Tyr Ser Lys-Ala Thr

Fig. 1 The amino terminal sequence of human P compared with previously published sequences of P (refs 2, 4), C1t (ref 7) and a cyanogen bromide fragment of CRP (ref. 7). Numbering and homology are based on the sequence of C1t. Amino acid sequence analysis was performed by using a Beckman 890C sequencer and the Beckman DMAA peptide program. Identification of amino acids was done by three methods: (1) gas–liquid chromatography on a 762 0A Hewlett-Packard gas chromatograph[8]; (2) thin-layer chromatography (TLC) of PTH-amino acids on 5 × 5-cm polyacrylamide plates in two dimensions[9]; (3) amino acid analysis using HI for hydrolysis[10]. Sequence identities are indicated by —. Unidentified residues by ( ). Residue 19 yielded tyrosine on TLC and histidine on back hydrolysis. In our conditions of amino acid analysis, PTH tyrosine is often lost and may elute with histidine.

and certain other substances (Y.L., I. Gigli, B.F. and E.C.F., in preparation), as well as further amino acid sequence studies will allow a more precise structural and functional comparison with C1t.

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An anterior pituitary factor stimulates thymidine incorporation in isolated thymocytes

A POSSIBLE tropic role of pituitary on thymus has been suggested by several studies. Hypophysectomy causes an atrophy of the gland[1-4] and thymus is poorly developed in Snellbagg genetically dwarf mice with hypopituitary function[5]. A diminution of thymus size and a reduction in immunological responses to T cell-dependent antigens is also seen after administration of anti-hypophyseal serum[6]. The pituitary may contain several active principles affecting directly or indirectly the thymus functions. Of these, growth hormone has been shown to have a promoting effect on metabolic activities of the thymus and on immune responses involving the participation of thymus-derived T cells[4,7,8]. Thyrotropin, via the thyroid hormones, also exercises a thymotropic action[9]. ACTH, via corticosteroids, causes the transient diminution of the gland[10]. We report here the presence in pituitaries of a previously unknown small molecular weight peptide(s) which stimulates markedly the incorporation of tritiated thymidine into DNA in isolated thymocytes.

Figure 1 shows that the addition of the anterior pituitary extract (APE) to the isolated thymocytes resulted in a notable stimulation of the incorporation of ^{3}H-thymidine into cold trichloroacetic acid (TCA)-precipitable pellet. A stimulation with APE was consistently obtained but the extent varied from preparation to preparation of APE and thymocytes. Thymocytes from young rodents gave a better response than those from aged animals. Table 1 shows the order of variation in stimulation by APE of ^{3}H-thymidine incorporation in thymocytes obtained from six different animals. Variance analysis of the data indicates that between-animal variations are significantly more marked than between tube variations (variance ratio $F = 29.9$, 8.5 times more than required for significance at 1% level). These variations probably arise due to different internal environments to which the thymocytes are exposed before the animals were killed. The effect of APE is highly significant ($F = 1163.5$) and is much more than the animal-to-animal variations (variance ratio 159.2 times higher than that required for 1% significance level).

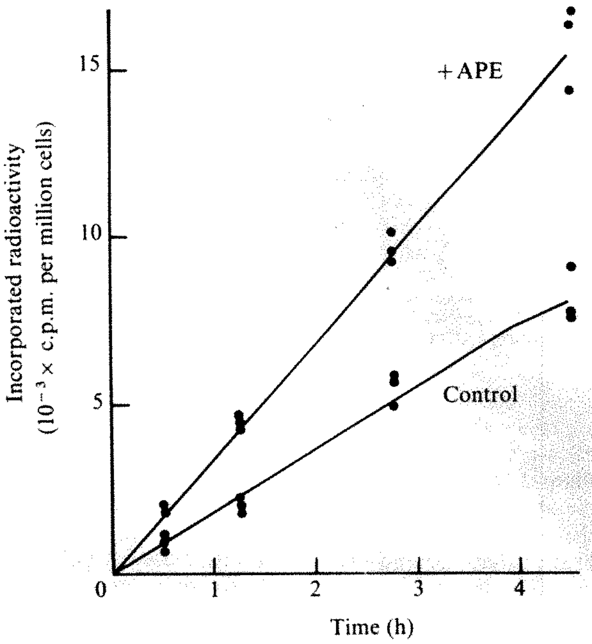

Fig. 1 Effect of anterior pituitary extract (APE) on ^{3}H-thymidine incorporation in isolated thymocytes. APE was the 2000*g* supernatant of rat anterior pituitary homogenates in GS medium (glucose salt medium, containing 120 mM NaCl, 5 mM KCl, 5 mM Na$_2$HPO$_4$, 1 mM MgSO$_4$, 0.8 mM CaCl$_2$, 5.5 mM glucose and 5 mM Tris-HCl buffer, *p*H 7.2). Rat thymocytes (15×10^6 ml^{-1}), were incubated in GS medium at 37 °C with ^{3}H-thymidine (0.5 μCi ml^{-1}, 2 μM) ± APE (added to a final concentration of 0.25 anterior pituitary equivalents per ml of final incubation medium). At different time intervals, the incorporation reaction was stopped by chilling and by adding excess (0.5 mM) of non-radioactive thymidine to the incubation tubes. Cells were centrifuged (600*g* for 10 min), washed with fresh GS medium, followed by two washings with cold 10% trichloroacetic acid and two with methanol. The pellet was dried and dissolved in 0.1 ml Soluene, transferred to the scintillation vials with a toluene-based scintillation mixture and the incorporated radioactivity measured in a Packard liquid scintillation spectrometer.

The incorporated radioactivity was sensitive to DNase but not to RNase treatment. Viability of cells was measured at each time point by Trypan blue exclusion and no significant difference was noted in control and APE added tubes.

To identify the pituitary hormones that may be responsible for this action of APE, rat growth hormones (NIAMD-rat GHB1), luteinising hormone (NIH-LH-S 17), follicle-stimulating hormone (NIH-FSH-S 9), thyrotropin (NIH-TSH-B 4), prolactin (NIH-P-B 3) and ACTH (pork ACTH, Chemical Lab. Friderikberg, Denmark) were tested singly and in combination. Two hormone combinations were prepared: in mixture A the hormones were present at their approximate physiological level in pituitary and in mixture B, at five times higher concentration. These hormones caused a marginal increase in thymidine incorportion, but the effect was not comparable with that obtained with APE (Table 2). The active principle in the extract thus seems to be different from these hormones.

The factor was dialysable and heat stable and was distinct from LH and TSH releasing hormones. The activity was not present in hypothalamic extracts or in posterior pituitary and cerebral cortex extracts. Cyclic nucleotides and inorganic ions did not give parallel stimulation of this metabolic activity in isolated thymocytes. The stimulatory effect was not due to the possible supply of essential

Table 1 Effect of anterior pituitary extract (APE) on ^{3}H-thymidine incorporation in thymocytes from six young rats

Rat	Body weight (g) and sex	Cell density in assay medium (million per ml)	Incorporated radioactivity c.p.m. per million cells ± s.e.m. Control	+ APE	% Of control
1	94.5 (F)	4.40	2652±133 (3)	7086±174 (4)	266.5
2	77.6 (F)	4.52	1832± 55 (4)	4483±210 (4)	244.7
3	74.3 (M)	5.12	2189± 28 (4)	5451±169 (4)	249.0
4	88.7 (F)	3.88	2964± 80 (4)	6553± 84 (4)	221.1
5	69.1 (F)	4.80	2824± 64 (4)	6041±295 (4)	213.9
6	44.1 (M)	1.76	2184± 42 (4)	6696±195 (4)	306.6

Thymocytes prepared separately from six young rats were incubated at 37 °C for 2 h ± APE (0.8 anterior pituitary equivalent per ml) in GS medium containing 1 μCi ml^{-1} ^{3}H-thymidine (1 μM). Values in parenthesis are the number of replicates for each preparation of cells.

nutrients by the APE, and was also obtained when experiments were performed in tissue culture media RPMI and Medium 199.

The stimulatory activity of the APE on thymocytes was sensitive to hog intestinal peptidase (from Sigma, containing general proteolytic and aminopeptidase activity) but not to DNase, RNase and trypsin. The factor may therefore be a peptide. Its molecular weight (MW) is 500, and the isoelectric *p*H 7.9. Purified preparations of the factor do not show significant ultraviolet absorption above 230 nm. These properties indicate that this factor is distinct from other factors of pituitary origin described in recent years by other investigators. Fibroblast growth factor from bovine pituitaries[12] has a MW of 13,300. Chondrocyte mitogenic factor[13] is a heat-labile entity and was detected as a contaminant in anterior pituitary hormone preparations. The factor influencing the ovarian cells in culture[14] is a non-dialysable protein. Lipotropins[15] are big polypeptides (6,000 and 11,400 molecular weight). A hypophysial factor influencing the steroid metabolism in rat hepatoma cell lines[16] is a high molecular weight entity and in contrast to present case, is present only in female pituitaries. Pituitary endorphins[17] are polypeptides of 3,000–3,500 MW.

The action of APE on uptake and incorporation of thymidine seems to be tissue specific. Thymocytes show maximal stimulation but lymphoid cells derived from spleen and lymph nodes are also stimulated, though to a lesser extent. APE did not have stimulatory effect on thymidine incorporation in tissue slices from liver, kidney, heart and diaphragm.

The biological role of this factor(s) is not fully known. It may influence the proliferation of cells within thymus. Mouse embryonic thymus cultures in media containing this factor show higher cellularity than parallel control cultures.

Thymus is an organ characterised by a high rate of cellular proliferation, which is independent of the antigenic stimulus[11], and not much is known on the factors regulating the metabolic activities and the rate of cell proliferation in this tissue. The size of this gland shrinks with hypophysectomy[1–4]. The gland is also atrophied in states of hypopituitary function[5]. It is thus conceivable that pituitary exercises an effect on lymphoid cell proliferation in thymus. The presence of a factor exerting a marked influence on DNA synthesis in thymocytes is thus of interest.

Table 2 Effect of anterior pituitary hormones and anterior pituitary extract on the incorporation of thymidine into isolated thymocytes

Addition	Incorporated radioactivity c.p.m. per million cells Mean ± s.e.m.	% Of control
None	429±31 (4)	100.0±7.2
Hormone mixture (A)	536±42 (6)	124.9±9.8
Hormone mixture (B)	584±57 (5)	136.1±13.3
Anterior pituitary extract (APE)	1567±95 (5)	365.3±22.1

Five million rat thymocytes in 1 ml GS medium, were incubated with tritiated thymidine (0.5 μCi ml^{-1}, 2 μM) with or without hormones/APE at 37 °C for 2 h in a Dubnoff metabolic shaker. Hormone mixture (A) contained at final concentration, 25 μg of GH, 20 μg each of TSH, FSH, LH and prolactins and 10 μg of ACTH per ml of incubation medium. Mixture (B) had five times the concentration of these hormones. APE was added at one rat anterior pituitary equivlaent per ml of final incubation medium. Figures in parenthesis give the number of replicates in each case.

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Propranolol increases binding of thyrotropin to thyroid membranes

β-ADRENERGIC antagonists such as propranolol possess two pharmacologically relevant properties—β-adrenergic blockade is observed at relatively low concentrations, whilst at higher concentrations they are effective membrane stabilising agents[1,2]. The latter property which, unlike β-adrenergic antagonism is non-stereospecific, is also described as local anaesthetic or quinidine like activity and it possibly accounts at least in part, for the anti-arrhythmic properties of propranolol—local anaesthetics and quinidine also showing anti-arrhythmic activity[3–5]. A wide variety of effects have been associated with local anaesthetic activity[1], but as yet there is no unifying explanation of the mechanism of action. We have investigated the effect of propranolol, acting in its capacity as a local anaesthetic, on a hormone receptor adenylate cyclase system which might be expected to be sensitive to membrane perturbation by local anaesthetics, and reported inhibition of thyrotropin (TSH) stimulation of adenylate cyclase in thyroid plasma membranes[6]. We present here a study of the consequence of local anaesthetic activity on binding of the hormone to membrane-associated receptors, and in this respect contrast the local anaesthetic activity of propranolol with that of local anaesthetics such as xylocaine or procaine.

^{125}I-TSH binds to plasma membranes derived from the thyroid in a reversible and specific manner, consistent with binding of the hormone to its physiologically relevant receptor linked to adenylate cyclase (see ref. 7 for review). Using experimental conditions previously described[8], we confirmed that ^{125}I-TSH bound to membranes prepared from the human thyroid can be significantly displaced by additions of the order of 25 μU of unlabelled TSH, and also by immunoglobulins derived from thyrotoxic sera[9], and have observed that relatively high doses of insulin, growth hormone, luteinising hormone and follicle-stimulating hormone produced no significant inhibition. As shown in Fig. 1, addition of D-propranolol (10^{-3} M) inhibited TSH stimulation of adenylate cyclase in human thyroid membrane preparations, but produced a highly significant increase in binding of the hormone to the membranes. The high concentrations of propranolol required to increase binding (10^{-3} M) and the equipotency of the D and L isomers of propranolol (not shown) together with the observation that quinidine (10^{-3} M) caused a similar increase, suggested that, as was previously reported for the inhibition of adenylate cyclase[6], the increased binding was attributable to the local anaesthetic properties of propranolol.

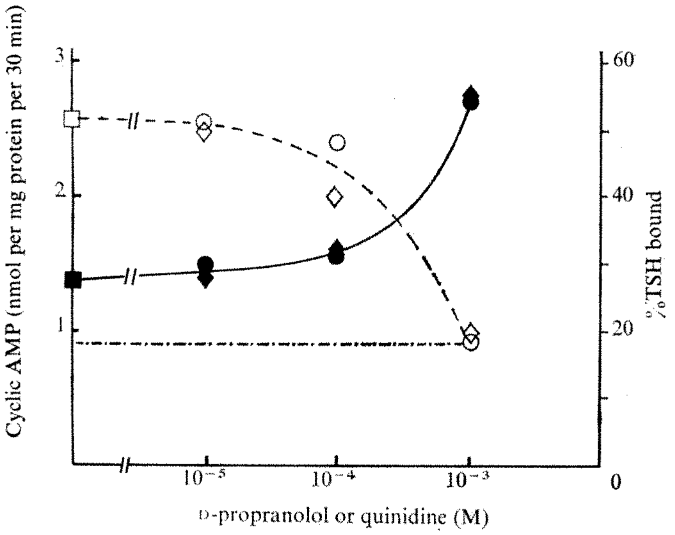

Fig. 1 The effect of propranolol and quinidine on TSH binding and stimulation of adenylate cyclase in human thyroid membranes. Membrane preparation and receptor purification of bovine ^{125}I-TSH were as described by Smith and Hall[8]. Binding was studied by incubating the membranes (50 μl at 2 mg protein per ml) with 200 μl iodinated bovine TSH (10,000 c.p.m. ml^{-1}) in the presence or absence of propranolol or quinidine, added as 50 μl of assay buffer (10 mM Tris-HCl, *p*H 7.5, 50 mM NaCl, 0.1% bovine serum albumin) with or without propranolol or quinidine (quinidine sulphate, Sigma). The incubation was terminated after 1 h at 37 °C by the addition of 1 ml of ice-cold assay buffer, the membranes then being separated by centrifugation (25,000*g*, 30 min at 4 °C), removal of the supernatant, and bound TSH determined by counting the membrane pellet. Stimulation of adenylate cyclase in the human thyroid membranes by bovine TSH (Armour, 1 U mg^{-1}) was as described before[6]. TSH (100 mU ml^{-1}) was added to membranes (2 mg protein per ml) in the presence of 2.5 mM ATP, 5mM Mg^{2+}, 10 mM theophylline, 10 mM creatine phosphate and 20 μg crystalline rabbit muscle creatine kinase, and the incubation terminated after 30 min at 37 °C. Cyclic adenosine 3′,5′-monophosphate was assayed by the method of Broan *et al.*[10]. TSH binding (means of duplicate determinations): ■, binding in absence of propranolol or quinidine; ●, binding in the presence of propranolol; ◆, binding in the presence of quinidine. TSH stimulation of adenylate cyclase (means of quadruplicate determinations): - - - - - -, basal, unaffected by propranolol or quinidine (10^{-3} M and less); □, TSH stimulated activity in absence of propranolol or quinidine; ○, TSH stimulated activity in presence of propranolol; ◇, TSH stimulated activity in presence of quinidine.

Table 1 The effect of β-adrenergic antagonists on binding of ^{125}I-TSH to human thyroid plasma membranes

Additions	% of ^{125}I-TSH bound (less nonspecific)
Experiment 1	
None	28.9±0.6
D-propranolol (10^{-3}M)	43.7±1.0
Oxprenolol (10^{-3}M)	32.5±0.4
Sotalol (10^{-3}M)	30.3±2.9
Alprenolol (10^{-3}M)	42.2±0.1
Experiment 2	
None	16.5±0.8
DL-propranolol (10^{-3}M)	57.4±2.2
Metoprolol (10^{-3}M)	20.7±0.9
Experiment 3	
None	22.8±1.4
D-propranolol (10^{-3}M)	53.5±0.7
Oxprenolol (10^{-4}M)	22.6±0.3
(10^{-3}M)	23.2±1.0
(5×10^{-3}M)	33.9±0.7
(10^{-2}M)	42.6±0.2
Practolol (10^{-5}M)	23.2±0.7
(10^{-4}M)	22.9±0.01
(10^{-3}M)	21.9±0.1

Experimental conditions used were as described in legend to Fig. 1. All drugs were in the form of the hydrochlorides, except metoprolol tartrate.

We investigated this further by comparing the effects of analogues of propranolol, all of which were β-adrenergic antagonists, but which varied in potency as local anaesthetics. When each drug was added to give a final concentration of 10^{-3} M, increased binding was observed in the presence of alprenolol, which possessed local anaesthetic potency comparable with propranolol and quinidine[11,12], but not following the addition of metoprolol, oxprenolol, sotalol or practolol, reported to exert comparatively little local anaesthetic activity[12,13] (Table 1). But, increasing oxprenolol concentrations to 10^{-2} M resulted in an increased binding which is consistent with its intermediate potency as a local anaesthetic[12].

Table 2 Effect of predicaine, carbocaine, xylocaine, procaine, and chlorpromazine compared with that of propranolol and quinidine on ^{125}I-TSH binding to human thyroid plasma membranes

Additions	% of ^{125}I-TSH bound (less nonspecific)
Experiment 1	
None	22.0±0.6
D-propranolol (10^{-3}M)	48.4±0.5
Quinidine sulphate (10^{-3}M)	43.7±1.2
Predicaine (10^{-3}M)	22.5±1.1
(10^{-2}M)	23.7±0.4
(10^{-1}M)	10.7±0.6
Carbocaine (10^{-3}M)	22.3±0.5
(10^{-2}M)	23.3±0.8
(10^{-1}M)	7.2±0.2
Xylocaine (10^{-3}M)	22.7±1.1
(10^{-2}M)	25.0±0.7
(10^{-1}M)	8.7±0.5
Experiment 2	
None	22.8±1.4
D-Propranolol (10^{-3}M)	53.5±0.7
Procaine (10^{-4}M)	21.1±0.8
(10^{-3}M)	23.0±0.2
(10^{-2}M)	20.4±0.5
(10–1M)	7.3±0.1
Experiment 3	
None	20.8±0.5
D-propranolol (10^{-3}M)	37.4±1.7
Chlorpromazine (10^{-5}M)	21.1±0.3
(5×10^{-5}M)	20.2±1.4
(10^{-4}M)	33.1±0.5
(10^{-3}M)	12.9±2.2

Experimental conditions were as described in the legend to Fig. 1.

Although increased binding of TSH apparently correlated with the local anaesthetic activity of β-adrenergic antagonists and quinidine, it is interesting that no increase was observed following the addition of the local anaesthetics xylocaine, predicaine, carbocaine and procaine. Detailed investigations, adding concentrations of xylocaine ranging from 10^{-7} to 10^{-1} M caused no increase in binding (not shown) and at concentrations of the order of 10^{-1} M, binding was reduced with xylocaine and the other local anaesthetics (Table 2). Another membrane stabilising agent, chlorpromazine, was found to increase binding when present at 10^{-4} M, although no such effect was reported by Moore and Wolff[14].

Scatchard analysis of the increased binding due to the presence of D-propranolol (10^{-3} M), suggests that it is due to an increase in apparent affinity of the hormone for the receptor, rather than an increase in the number of receptors (Fig. 2). Explaining the nonlinear plots as due to the presence of a high- and low-affinity binding sites, they may be interpreted to indicate an increase in affinity of the high-affinity site from 2.5×10^9 l mol^{-1} to 8×10^9 l mol^{-1} in the presence of propranolol.

The detailed mechanism by which propranolol might increase the affinity of the receptor for TSH is unknown, but evidence from several investigations suggests that it may be primarily due to changes in the hydrophobic lipid environment of the membrane-associated receptors. A correlation between increased local anaesthetic potencies of β-adrenergic antagonists and an increase in their hydrophobic nature has been recognised[12], and increased binding of TSH to thyroid membranes has also been reported in the presence of filipin and following membrane treatment with phospholipase A (ref. 14). Moreover, a sharp increase in binding of TSH observed at temperatures of 30 °C and above has been associated with increased fluidity of membrane lipids, as characterised by motional characteristics of three membrane bound fluorescent probes[15]. It is well recognised that TSH stimulation of adenylate cyclase in membranes is sensitive to the presence of phospholipids[16] and the observation that local anaesthetics interact specifically with anionic phospholipids[17] might account for the effect of D-propranolol on the TSH stimulation[6,18].

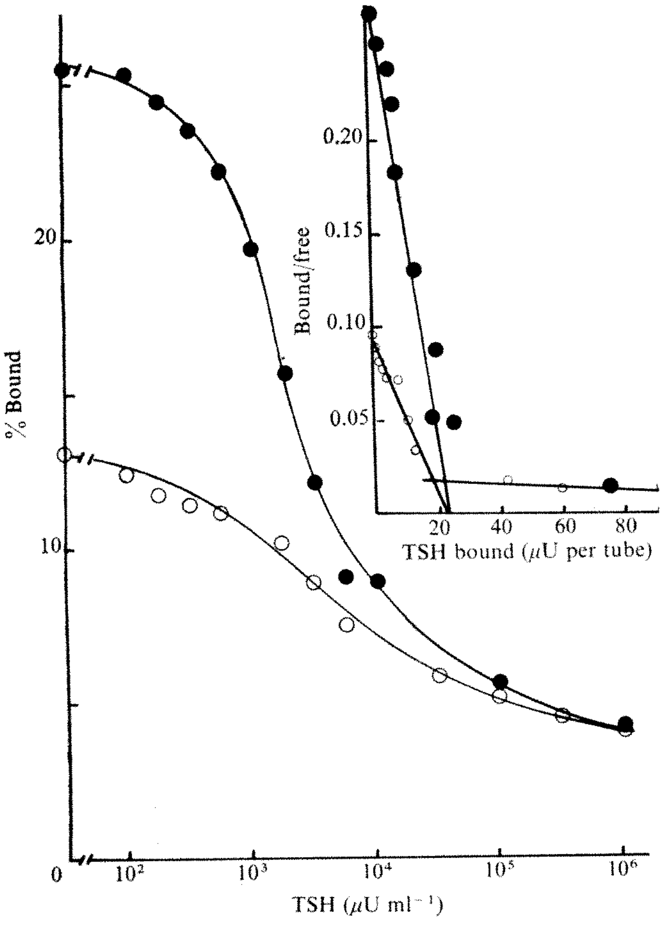

Fig. 2 The effect of propranolol on the binding of bovine ^{125}I-TSH to human thyroid membranes in the presence of additional unlabelled bovine TSH. Experimental conditions were as described in the legend to Fig. 1, and unlabelled TSH (Armour, 1 U mg^{-1}) was added at the concentrations shown. The Scatchard plot of these results is shown inset. ○, In the absence of 10^{-3} M D-propranolol; ● in the presence of 10^{-3} M D-propranolol.

Local anaesthetic activity of β-adrenergic antagonists might also be expected to be effected by local anaesthetics such as xylocaine, and it is significant that despite the correlation between the local-anaesthetic properties of β-adrenergic antagonists and their ability to increase binding, a similar effect was not observed with the local anaesthetics of either the amide or ester type included in this study.

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Polypeptides similar to the α and β subunits of tubulin are exposed on the neuronal surface

THE finding by Borisy and Taylor[1] that nervous tissue was the source of large amounts of soluble tubulin, the subunit protein of microtubules, has greatly facilitated the biochemical study of microtubular structure. From 10% to 20% of the total protein of developing brain is in the form of soluble tubulin[2,3] and most studies have focused on this form of the protein. In 1970, however, Feit and Barondes demonstrated a large amount of colchicine binding activity, a term which has become associated with the presence of tubulin, in particulate fractions of brain[4]—fractions expected to be free of microtubule-derived tubulin. Recent studies indicate that polypeptides with the same size and drug-binding properties as soluble tubulin are present in synaptosomal plasma membranes[5–7]. These studies suggest a membrane-associated form of the protein which is insoluble in non-chaotropic buffers. I here report experiments which provide additional evidence for membrane-associated tubulin-like proteins and also suggest that a portion of this polypeptide(s) may be exposed on the external surface of the cell.

Superior cervical ganglia from perinatal rats were separated into three to four pieces and cultured on collagen-coated dishes. The general properties of this nerve cell in culture have been reviewed[8,9]. These explants were treated with a combination of antimitotic drugs, in a regimen modified from that used on dorsal root ganglion by Wood and Bunge[10], in order to suppress all cellular growth except that of the neurones. The result of three weeks' growth of four explants in one culture is shown in Fig. 1. The neurones within the explant produce copious neurites, up to 5 mm in length and the dish contains essentially no non-neuronal cells. Such cultures were used to isolate samples of neurites free of their cell bodies and of non-neuronal cells by simple excision and removal of the explant regions from the dish, with subsequent harvest of the extensive neurite outgrowth.

If such explants are given radioactively labelled amino acids in their culture medium, it is possible to demonstrate, by autoradiographic analysis of SDS–polyacrylamide gels of neuronal polypeptides, that the synthesis of tubulin and actin account for as much as one-third of total protein synthesis (ref. 11 and my own data, not shown). Furthermore, since the neurites are metabolically dependent on the cell somata for polypeptides needed in their growth and maintenance, it is possible to study the transport of protein from the somata into the neurites. In pulse-chase experiments of this type, polypeptides co-migrating with tubulin are among the most prominent seen by fluorography (Fig. 2).

When a similar experiment is carried out using fucose as a precursor, polypeptides co-migrating with the tubulin subunits are again heavily labelled (Fig. 3). Comparison of soluble and particulate fractions from these neurites reveals that little or no fucose-derived radioactivity is associated with the soluble tubulin but that the particulate fraction contains heavily labelled polypeptides co-migrating with tubulin.

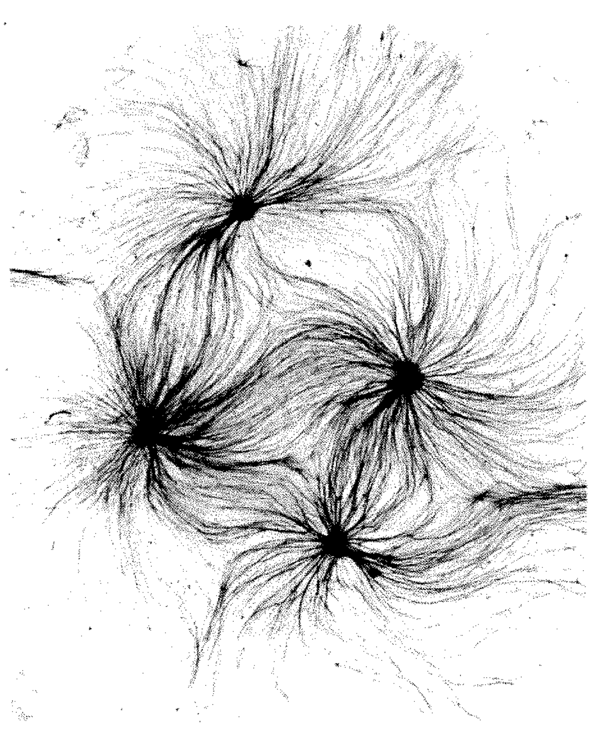

Fig. 1 Photomicrograph of superior cervical ganglion explants after 3 weeks' growth in culture. Cultures were fed with Eagle's minimal essential medium (80 vol. %), supplemented with glucose (600 mg %), human serum (10 vol. %), 150 mM KCl (10 vol. %), and nerve growth factor (20 ng ml^{-1}). The media contained 10^{-5} M 5′-fluorodeoxyuridine continuously, and 10^{-5} M cytosine arabinoside from day 2 until day 7, *in vitro*. The neuronal cell bodies are confined within the explants and the neurites, free of supporting cells, extend from each explant. Before neurite collection, the explants were removed by dissection. (MX5.)

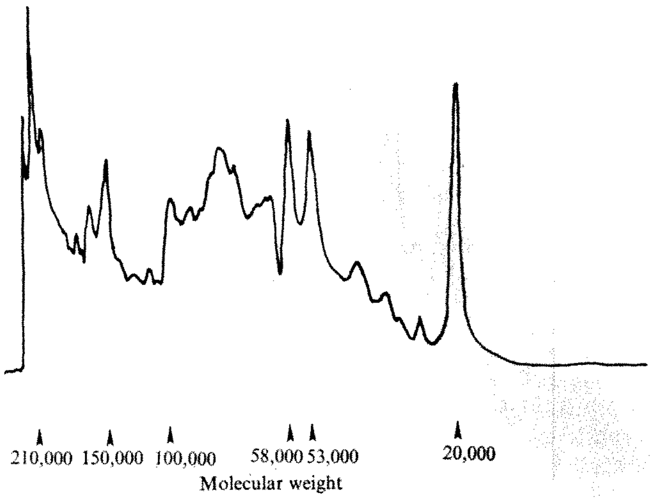

Fig. 2 Densitometric scan of a fluorograph made from contact with an SDS slab gel. The sample was composed of neurites collected from explants which had been exposed to a 2-h pulse of radioactive leucine (20 μCi ml^{-1} in medium made with leucine-free MEM and three times dialysed human serum). The polypeptides were separated by SDS-discontinuous slab gel electrophoresis on a 5–15% gradient of polyacrylamide. These radioactive polypeptides had been transported into the neurites after synthesis in the neuronal somata. The two large peaks of radioactivity at molecular weight 53,000 and 58,000 co-migrate with the α and β subunits of tubulin. The absence of a labelled peak co-migrating with actin is explained by the observation that actin is transported at a slower rate than tubulin and at this moment, has not yet entered the neurite in substantial amounts. Electrophoresis and fluorography were as described in refs 12 and 18. Molecular weight standards and assigned molecular weights were: phosphorylase A (94,000), bovine serum albumin (68,000), catalase (60,000), glutamic dehydrogenase (56,000), IgG, heavy chain (50,000), ovalbumin (43,000), aldolase (40,000), lactate dehydrogenase (36,000, α-chymotrypsinogen 25,000), IgG, light chain (23,500), myoglobin (17,200). Porcine brain tubulin was used as standards for α and β tubulin.

This experiment suggested that polypeptides similar to the α and β subunits of soluble tubulin were associated with the neuritic plasma membrane. In order to investigate the polypeptides of the plasma membrane, external labelling using iodination catalysed by glucose oxidase/lactoperoxidase was attempted. The results of such experiments are shown in Fig. 4. A relatively small number of polypeptides became labelled in conditions in which the enzymes should have remained outside the neurites (Fig. 4*a*, *c*). That the labelling pattern is due to iodination of polypeptides exposed on the cell exterior is supported by a number of control experiments: (1) the labelling is entirely dependent on the presence of the catalytic enzymes; (2) the labelling pattern which homogenised neurites exhibit is much more inclusive, with essentially all of the polypeptides demonstrated by Coomassie blue staining labelled. The most striking example of this is the heavy labelling of polypeptides co-migrating with actin, when the iodination is done on homogenised neurites as compared to the absence of label in that region after iodination of intact neurites (experiment not shown); (3) none of the media polypeptides or substrate polypeptides show an iodination pattern similar to that in Fig. 4, when examined electrophoretically. This latter control suggests that the surface-associated tubulin-like proteins are not derived from polypeptides present in the medium.

The effects of trypsin treatment on the iodination pattern further support the hypothesis that the tubulin-like polypeptides are exposed at the exterior surface of the neuronal plasma membrane. Figures 4*d* and *e* show that brief treatment with low levels of trypsin will remove the iodinatable species which co-migrate with soluble reference tubulin. This suggests a

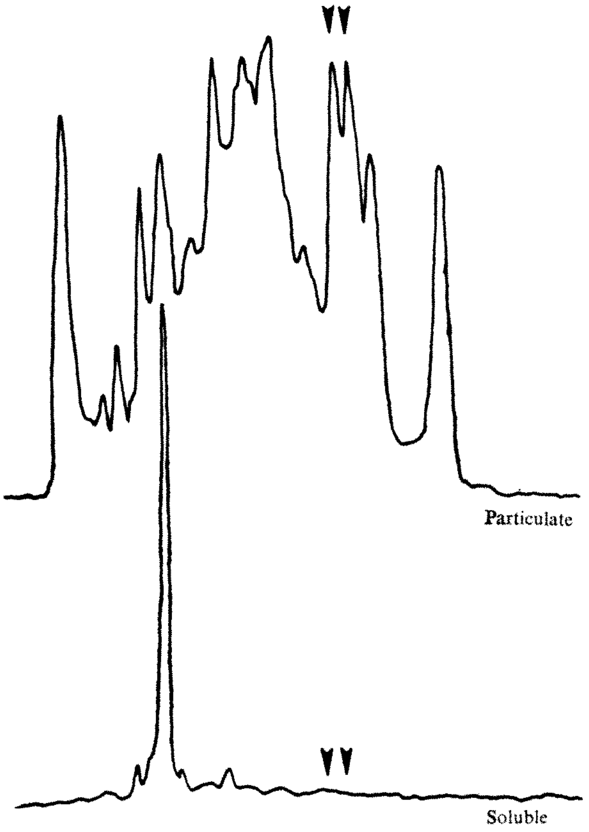

Fig. 3 Densitometric scans of fluorographs made from contact with SDS-slab gels. The samples were derived from neurites harvested from explants after 12 h of labelling with tritiated fucose (10 μCi ml^{-1} in medium made with three times dialysed serum). In the upper scan, the sample consisted of insoluble neuritic material after homogenisation in 150 mM NaCl, 5 mM sodium phosphate (*p*H 7.2), and centrifugation at 100,000*g* for 1 h. The lower scan represents the soluble neuritic material derived from the same homogenate. In the lower scan, there is no radioactivity associated with the area of the gel containing the α and β subunits of tubulin (arrows). In the particulate sample, however, polypeptides co-migrating with α and β tubulin subunits are substantially labelled. Direction of electrophoresis was from left to right. Electrophoresis and fluorography as in Fig. 2.

restricted amount of the iodinatable species, most or all of which is removed in the proteolytic treatment. Data not shown demonstrate that there are no differences in the Coomassie blue staining patterns of the samples used in Fig. 4*c*, *d*, *e* indicating the small amount of proteolysis which actually occurs during such treatment, and more importantly, that the bulk of the neuritic tubulin is unaffected while the co-migrating label is removed.

In the Laemmli SDS gel system[12], the α and β subunits of soluble tubulin are resolved and migrate at molecular weight 58,000 and 53,000, respectively. If the ionic strength of this same buffer system is increased by the addition of 300 mM NaCl, the tubulin subunits co-migrate[13]. When aliquots of the same sample of iodinated neurites are run in the two gel systems, the labelled polypeptides in the molecular weight range 50,000–60,000 always co-migrate with soluble reference tubulin subunits no matter whether the reference tubulin subunits resolve (as in the regular Laemmli system) or not (as in the high ionic strength modification). Thus, in two quite different gel systems, these two polypeptides behave similarly to soluble tubulin.

Taken together, these experiments strongly suggest and strengthen the view that tubulin-like polypeptides are present in the neuronal plasma membrane and further, that these polypeptides may be exposed on the exterior of the cell surface.

The role of such a protein may only be hypothesised at this time. It is well known that a colchicine-sensitive mechanism can control the mobility of polypeptides in the cell membrane[14]. These membrane-associated tubulin-like polypeptides may act as anchors for the membrane-associated microtubules described by a number of electron microscopists. In terms of neuronal cell biology, Levi-Montalcini *et al.* have presented evidence that a tubulin-like molecule may have a role in the recognition of nerve growth factor (NGF) at the cell surface[15]. In their study, live intact mouse neuroblastoma cells, which had NGF receptors on their surfaces, would form rosettes with

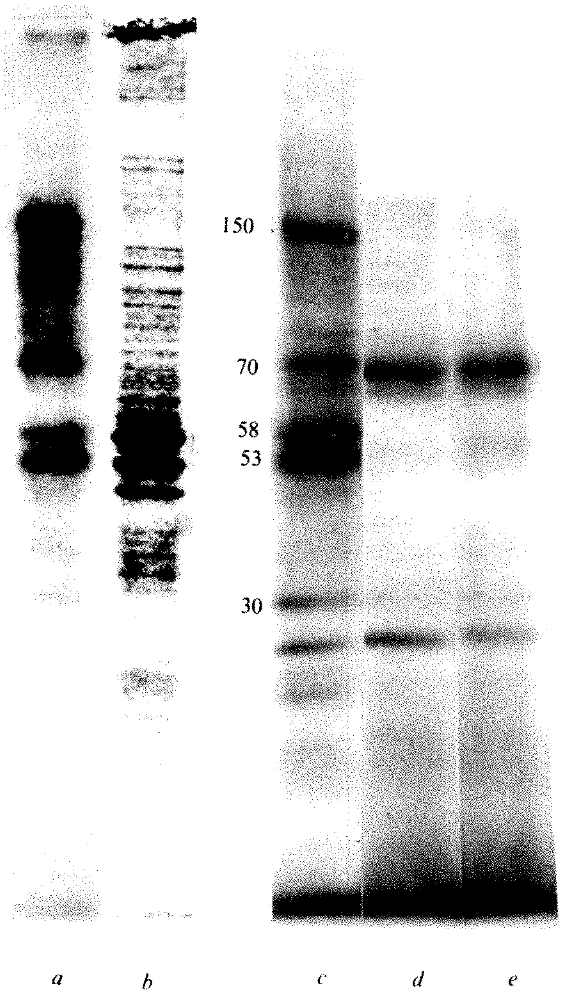

Fig. 4 Results of iodination experiments on intact neurites isolated from superior cervical ganglion explants. Panels *a*, *c*, *d*, *e* are autoradiographs of SDS-slab gels. Panel *b* is a picture of a Coomassie blue stained gel which produced the autoradiograph shown in panel *a*, and allows for molecular weight assignments of the iodinated bands. All samples consisted of neurites which had been iodinated using 5 milliunits ml^{-1} each of lactoperoxidase and glucose oxidase, 5 mM glucose, and 100 mCi ml^{-1}. Na^{125}I, in phosphate-buffered saline, *p*H 7.2. After 15 min labelling at 0 °C, samples were repeatedly washed with PBS containing excess iodide. Sample *d* consisted of neurites which had been treated with 20 μg ml^{-1} crystalline trypsin for 15 min at room temperature before iodination. Sample *e* consisted of neurites which had been treated with the same amount of trypsin after the iodination procedure. Samples *a* and *c* consisted of non-trypsin treated, control iodinated neurites. Note that the protease treatment removed the label at molecular weight 53,000 and 58,000, which co-migrated with α and β tubulin. Also removed by protease treatment were prominently labelled polypeptides migrating at molecular weight 150,000 and 30,000. In sample B, the prominent polypeptide band below the heavy bands at molecular weight 53,000 and 58,000 co-migrated with purified muscle actin. Note the absence of any iodine incorporation into this major polypeptide in the autoradiographs of the iodinated neurites. The numbers in the centre of the figure indicate approximate molecular weight $\times$ 10^{-3}, as determined by comparison with polypeptides of known molecular weights. Electrophoresis was as described in ref. 12. Autoradiography was accomplished by exposing Kodak RPR-54 film to the stained and dried slab gel.

NGF-coated red cells. This rosette formation presumably represented the hormone–receptor interaction. Antibody prepared against soluble brain tubulin could prevent rosette formation in their experiments.

Actin and myosin have now been shown to be associated with the surface membrane in a variety of non-muscle cells[16,17]. The identification of tubulin in the surface membrane of neurones suggests that it will be necessary to expand our ideas of the roles this protein may play in cell function.

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Type I and type II cyclic AMP-dependent protein kinase as opposite effectors of lymphocyte mitogenesis

THE role of cyclic AMP in the regulation of cell growth and differentiation has been extensively investigated in many cell types following various growth stimuli[1,2]. In recent years, peripheral blood lymphocytes treated with antigens[3], plant lectins[4], lipopolysaccharides[5], or periodate oxidation[6] have served as a model system for assessing whether cyclic AMP is involved in the regulation of the mitogenic response promoted in these cells by the above classes of agents[7,8]. There are two conflicting hypotheses, as outlined by Friedman[1], concerning the role of cyclic AMP in lymphocyte proliferation. The first hypothesis suggests that the increase in cyclic AMP levels following mitogen stimulation[8] and the subsequent increase in ^{32}P incorporated into Fl histones[9] and certain cystosol proteins[10] implicates cyclic AMP as a positive mediator of mitogenesis in lymphocytes. The other hypothesis reasons that cyclic AMP inhibits proliferation, and this hypothesis is supported primarily by observations from several laboratories that raising the intracellular cyclic AMP level through the use of phosphodiesterase inhibitors[11], prostaglandins[12], or cyclic AMP analogues[11,13–15] effectively inhibits mitogen-stimulated RNA and DNA synthesis. We present here evidence that may help resolve this apparent paradox. Incubation of Ficoll–Hypaque purified human peripheral blood lymphocytes with concanavalin A (conA) leads to the activation of only type I cyclic AMP-dependent protein kinase in these cells even though there are significant amounts of both type I and type II kinase present. But, the addition of dibutyryl cyclic AMP (DBcAMP) to the conA-stimulated lymphocytes at a concentration sufficient to block the synthesis of RNA and DNA results in the activation of both type I and type II cyclic AMP-dependent protein kinase. It therefore seems likely that while the activation of type I protein kinase represents a positive component in the progression of events promoted in a lymphocyte by a mitogenic signal (as it does in other cell types in which a trophic response is evoked), the activation of type II protein kinase (or perhaps types I and II in concert) represents a mechanism by which a negative influence can be imposed on the pro-

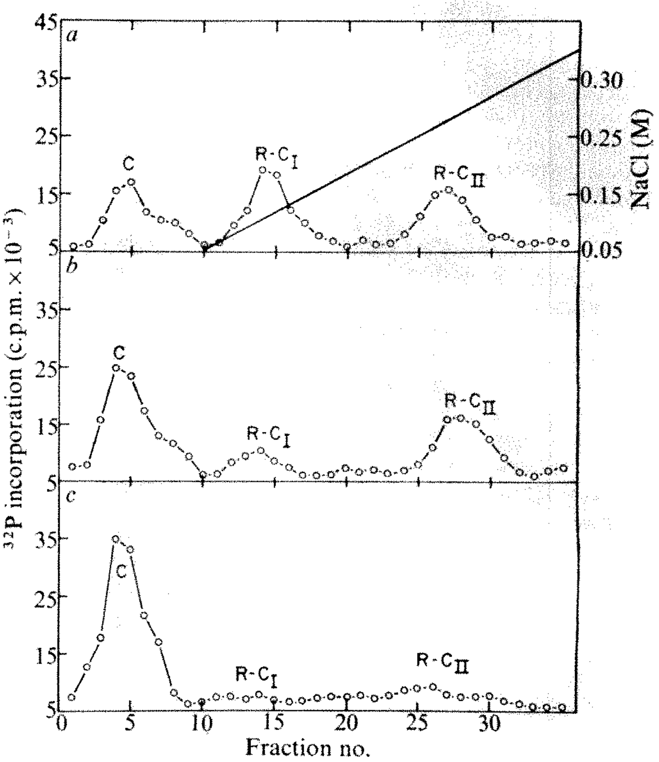

Fig. 1 Type I and type II protein kinase activation following con A and DBcAMP administration. Human peripheral blood lymphocytes were isolated from heparinised venous blood from healthy male donors by flotation on Ficoll–Hypaque (Litton Bionetics) and washed three times using phosphate buffered saline as described previously[23]. The cells were suspended in 10 ml of Eagles medium at a density of 2 × 10^6 per ml and preincubated at 35 °C overnight. Con A was added at 10 μg ml^{-1} resulting in maximal rates of RNA and DNA synthesis at 48 and 72 h respectively. Previous experiments using supernatant determinations of the without cyclic AMP/with cyclic AMP protein kinase activity ratio (data not shown) indicated that total cyclic AMP-dependent protein kinase activation was greatest after a 4-h incubation with con A. DBcAMP 10^{-5} M when added concomitant with con A blocked by greater than 90% the increase in ^{3}H-uridine and ^{3}H-thymidine incorporation into acid-precipitable material normally seen at 48 and 72 h. C^6-amino alkyl Agarose chromatography of unstimulated (*a*), con A (*b*), and con A and DBcAMP (*c*) stimulated lymphocytes (4-h incubations) was performed as described by Rangel-Aldao and Rosen[16] with modifications. The C^6-Agarose was prepared according to Shaltiel[25] and 1-ml columns of resin were equilibrated with 15 mM sodium–potassium phosphate, *p*H 6.8, 1 mM EDTA, 0.5 mM methylisobutylxanthine, 5 mM β-mercaptoethanol, 5 mM NaFl, and 60 mM NaCl. This salt concentration was sufficient to allow the free catalytic subunit to pass through the column while the regulatory subunits and type I and type II holoenzyme were bound. The capacity and the required initial salt concentration was determined with purified C, R_IC (type I), and $R_{II}C$ (type II) for each batch of resin. 60 × 10^6 cells were sonicated for 10 s in 300 μl of the above buffer at 0 °C and 250 μl of the sonicate applied to the column. The column was rapidly washed for 10 fractions (200 μl per fraction) with buffer containing 60 mM NaCl and a gradient from 60 to 350 mM NaCl (4 ml of each) begun. Aliquots (50 μl) of each fraction were assayed for kinase activity with mixed calf thymus histone as substrate in the presence of 5 μM cyclic AMP (ref. 19). Purified regulatory and catalytic subunits showed no reassociation when chromatographed in these conditions.

liferative process by the generation of abnormally high levels of intracellular cyclic AMP.

Human peripheral blood lymphocytes were purified and cultured as described in the legend to Fig. 1. Using C^6-amino alkyl Agarose chromatography, originally used by Rosen and coworkers to study isozymes of cyclic AMP-dependent protein kinase [16,17], the two isozyme forms (R_IC and $R_{II}C$, inactive holoenzyme forms) were rapidly separated from each other and from the dissociated free catalytic subunit (C), that is, activated protein kinase. Con A addition, which promotes an increase in cyclic AMP (ref. 8), activates only type I of the kinase. Fig. 1*a*, *b* shows that kinase activity decreases in those fractions containing type I holoenzyme and appears in the flow-through fractions which correspond to free active catalytic subunit. Addition of DBcAMP to the con A-stimulated lymphocytes further reduces kinase activity from the elution profile which corresponds to type II holoenzyme and again the activity is recovered as catalytic subunit in the flow-through fractions (Fig. 1*c*).

The mechanism by which the activation of type II kinase by DBcAMP in lymphocytes might lead to inhibition of mitogen-induced proliferation is not immediately apparent. However, the activation of both forms of the kinase rather than the selective activation of type I at a time when the cell is preparing to synthesise protein, RNA, and DNA in a specific series of events leading to eventual cell division may interfere with any of a multitude of parameters mediating this process. These could include Ca^{2+} fluxes, ribosome aggregation, cyclic GMP events, and/or transport of precursors or essential serum factors from the media.

It is becoming increasingly evident that type I and type II cyclic AMP-dependent protein kinase may have separate functions in the cell. They have specific expression patterns during cell cycle[18], cardiac hypertrophy[19], and during the growth and differentiation of certain tissues[20]. Lee *et al.*[20] have shown that during testicular ontogeny, type I protein kinase is present and the appearance of differentiated cell types in the testis is concomittant with an increase in type II protein kinase. In addition, the two forms of protein kinase have different regulatory properties. Rosen[16] has shown that only the regulatory subunit of type II holoenzyme is phosphorylated, greatly reducing its ability to reassociate with free catalytic subunit. A separate role was postulated for this isozyme based on this unique phosphorylation event. In those cell culture cells in which cyclic AMP events have been studied, cyclic AMP increases markedly during G_1 phase of the cell cycle, decreases at the G_1/S border, and increases again in G_2/M. Addition of DBcAMP and a phosphodiesterase inhibitor early in G_1 prevents Chinese hamster ovary cells from progressing from G_1 into S phase[21]. In fact, it also results in differentiation as assessed by cell elongation and increased microtubular structures[21,22].

Therefore, while activation of type I cyclic AMP-dependent protein kinase represents a positive component in events promoted in the lymphocytes by a mitogenic signal, the activation of type II protein kinase (or perhaps types I and II in concert) represents a mechanism by which a negative influence can be imposed on the proliferative response by the generation of an abnormally high intracellular cyclic AMP concentration. This study for the first time points to the complexity of cyclic AMP mediation, since it acts through two specific enzymes regulated by alterations in cyclic AMP levels.

Further studies of tissues at various stages of differentiation in regard to pools of protein kinase, type I and type II, as well as the activation patterns of these kinases after intracellular changes in cyclic AMP in response to hormones which are specific stimuli and to the addition of cyclic nucleotide analogues and phosphodiesterases which are nonspecific stimuli are necessary to ascertain if these parameters operate in the control of growth and differentiation.

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Chinese hamster cell mutant with defective mitochondrial protein synthesis

STABLE mutations affecting mitochondrial functions have been reported in lower eukaryotes[1–7] and higher organisms[8–11]. We have described a procedure to select mutants with defective patterns of oxidative energy metabolism from established Chinese hamster fibroblasts[8], which seem to satisfy most of their energy needs from glycolysis[6]. We describe here a mutant which has properties reminiscent of the petite phenotype of yeast ρ^- mutants[5,13,14]: a defect in mitochondrial protein synthesis leads to multiple enzymatic deficiencies.

The mutant cells, V79-G7, were characterised as respiration-deficient by the following criteria[8–10]. (1) The rate of oxygen consumption by whole cells is about 10% that of wild-type cells. (2) There is no appreciable release of $^{14}CO_2$ from 2-^{14}C-pyruvate or 5-^{14}-C-glutamate (conversions requiring an active and complete Krebs cycle). (3) The cells behave as auxotrophs for CO_2 and asparagine, compounds normally derived from the Krebs cycle. And (4), when galactose, a slowly fermentable carbon source, is substituted for glucose in the medium, the cells die overnight. In these respects V79-G7 cells resemble all other respiration-deficient fibroblasts isolated from the parental line[8]. A unique property of this mutant was a reduced capacity of isolated mitochondria to respire in the presence of succinate or α-glycerolphosphate. Succinate dehydrogenase activity in mitochondria was much less reduced (Table 1). These results focused our attention on the electron transport chain between coenzyme Q and oxygen.

The terminal enzyme complex of the electron transport chain is cytochrome oxidase which can be assayed in purified mitochondria using exogenous, heterologous, reduced cytochrome *c* as substrate[15]. In Fig. 1 a rate constant,

Table 1 Biochemical activity of V79 and V79-G7 mitochondria

	Activity per mg protein V79	V79-G7	% of control
Succinate oxidation	1180 ± 120 nmol O_2 h^{-1}	400 ± 90 nmol O_2 h^{-1}	33.9 ± 7.6
α-glycerol phosphate oxidation	1320 ± 190 nmol O_2 h^{-1}	700 ± 40 nmol O_2 h^{-1}	53.0 ± 3.0
Cytochrome oxidase activity	24.4 ± 12.3 ml min^{-1}	1.6×0.8 ml min^{-1}	6.6 ± 3.3
ATPase activity (oligomycin-sensitive)	380 ± 56 nmol P_i h^{-1}	44 ± 11 nmol P_i h^{-1}	11.6 ± 2.9
Succinate dehydrogenase	23.7 nmol mg^{-1}	17.0 nmol min^{-1}	72

Assays of selected activities of isolated mitochondria. Cells were grown in Dulbecco's modified Eagle's medium (DME) with the following additions: 45 μg ml^{-1} asparagine, 4 mg ml^{-1} glucose, 20 mM HEPES buffer, *p*H 7.5, 10% foetal calf serum (Irvine Scientific) and alternating antibiotics. Mitochondria were isolated following the procedure of White and Tewari[25]. Washed cells were homogenised in a Dounce homogeniser in buffer containing 250 mM sucrose, 2 mM EDTA, 50 mM Tris, *p*H 7.4 (SM buffer), containing 1% bovine serum albumin (BSA). After removal of the nuclei the mitochondria were pelleted in the SS34 Sorvall rotor (10,000 r.p.m., 10 min) washed once by resuspension in SM buffer plus 1% BSA and repelleted as above. All manipulations were carried out at 0–4 °C. For the measurement of oxygen consumption mitochondria were resuspended in 2 ml SM buffer plus 1% BSA, placed in a thermostated oxygen electrode chamber, and oxygen consumption was measured as described previously[9]. The cytochrome oxidase assay is described in the legend to Fig. 1. The oligomycin-sensitive ATPase was found to be most reproducibly measured after treatment of the mitochondria at *p*H 9.2, in SM buffer, at room temperature[26]. 100 μl of mitochondrial suspension was then mixed with 350 μl of Tris buffer (25 mM, *p*H 9.2) containing Mg^{2+} (3.3 mM), plus or minus oligomycin (44.4 μg ml^{-1}), and finally 50 μl of ATP (50 mM) was added to the mixture. After 10 min at 30 °C the reaction was terminated by addition of trichloroacetic acid (final concentration 10%) and inorganic phosphate determined by the method of Fiske and Subbarow[27]. Approximately 60% of the total ATPase activity observed in wild-type mitochondria was inhibitable by oligomycin. Mg^{2+} ions were essential for all observed activities.

assumed to be proportional to cytochrome oxidase activity is plotted against amounts of Lubrol added to the mitochondrial preparation. Treatment of wild-type mitochondria with detergent uncovered measurable activity, whereas in the mutant, enzyme activity remained very low, at most 6.6% relative to wild type.

These results were confirmed by measurements of the absorbance spectra of fully reduced whole cells in the wavelength range 400–630 nm (Fig. 2). The most notable differences between mutant and wild-type cells were observed at 440 nm and at 600 nm corresponding to the Soret band and the α band, respectively, of the cytochromes *aa*$_3$. Cytochromes *aa*$_3$ seem to be absent in the mutant cells. Although uncertainties of the baseline make quantitative comparisons difficult, there seems to be no decrease in the cytochrome *c* content of the mutant cells, as judged by the absorption peak at 550 nm. But, the shoulder at 560 nm, the region of absorption of cytochrome *b*, seems to be reduced in the case of the mutant cells.

From work done in yeast and *Neurospora*, it is known that the cytochrome oxidase and cytochrome *b* contain subunits which are synthesised inside the mitochondria, whereas cytochrome *c* is synthesised exclusively in the cytoplasm[13,14,16,17]. Another enzyme known to have several subunits synthesised within the mitochondria is the oligomycin-sensitive ATPase (refs 13, 14, 18). We assayed this enzyme in mitochondria and found that its activity, like that of cytochrome oxidase, was greatly reduced in the mutant cells (11.6% relative to wild type, see Table 1).

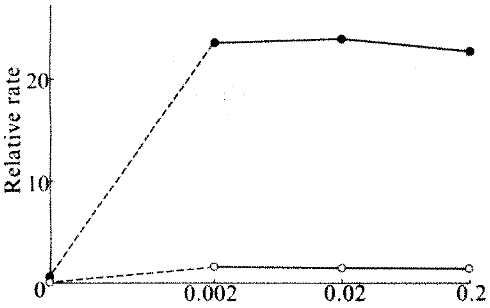

Fig. 1 Cytochrome oxidase activity in detergent-treated wild-type and mutant mitochondria. After a 10-min pretreatment of the mitochondria in SM buffer with various concentrations of Lubrol the reactions were initiated by addition of excess reduced cytochrome *c* from beef heart[12] and the absorbance was measured at 550 nm with a recording spectrophotometer. Experiments were performed at 25 °C. The observed rate constants were normalised to the same concentration of total mitochondrial protein in the reaction mixture. Absolute rates are given in Table 1.

The properties of the mutant described so far, and the similarity to the phenotype of the ρ^- mutants of yeast, prompted us to examine mitochondrial protein synthesis *in vivo* in wild-type and V79-G7 cells. In these experiments cytoplasmic protein synthesis was inhibited by cycloheximide, and the cells were then labelled with ^{3}H-leucine, followed by a chase with cold leucine. In wild-type cells cycloheximide, at 100 μg ml^{-1}, inhibited total incorporation of ^{3}H-leucine into whole cells by 95–97%. Purified mitochondria were dissolved in buffer containing SDS and β-mercaptoethanol, and their proteins were fractionated electrophoretically on sodium dodecyl sulphate–polyacrylamide slab gels (Fig. 3). The wild-type pattern shows at least seven peaks of radioactivity, corresponding to a range in size from 10,000 to 44,000 MW (Fig. 3*a*). In contrast, an equivalent amount of mitochondrial protein from the mutant cells gave no such distinctive profile (Fig. 3*b*). Only traces of the 22,500 and 25,000 peaks were visible. The amount of ^{3}H-leucine incorporated per milligram of protein of the mitochondrial fraction was slightly greater than one-third of that in wild-type cells. Most of the radioactivity

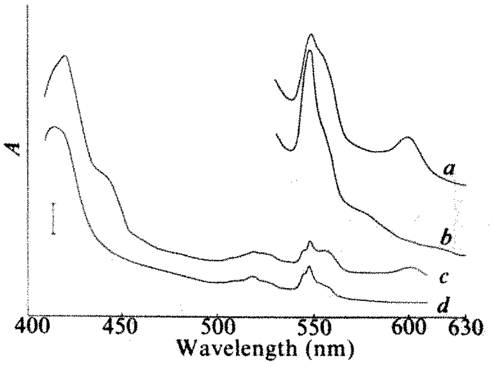

Fig. 2 Absorption spectra of reduced, whole cells. A suspension of cells in buffer containing NaCl (137 mM), KCl (5 mM), Na_2HPO_4 (0.7 mM), Tris (25 mM), *p*H 7.3, was reduced by addition of a few grains of dithionite and then frozen in quartz spectrophotometer cuvettes at a density of 10^7 cells per ml. Absorption spectra were recorded at liquid nitrogen temperature in a Cary 14 spectrophotometer, with the output from the photomultiplier fed into a PDP 8/1 computer for the summation of multiple scans over the wavelength region indicated. Two independent experiments are shown: wild-type (*c*) and mutant cells (*d*) were scanned from 410 to 610 nm, and the experiment was repeated with different batches of cells: wild-type (*a*) and mutant cells (*b*), with a scan from 530 to 630 nm. Approximately equal numbers of wild-type and mutant cells were compared in each experiment. The bar represents an absorbance change of 0.1 (curves *c* and *d*), and the scale is changed by a factor 4 for curves *a* and *b*.

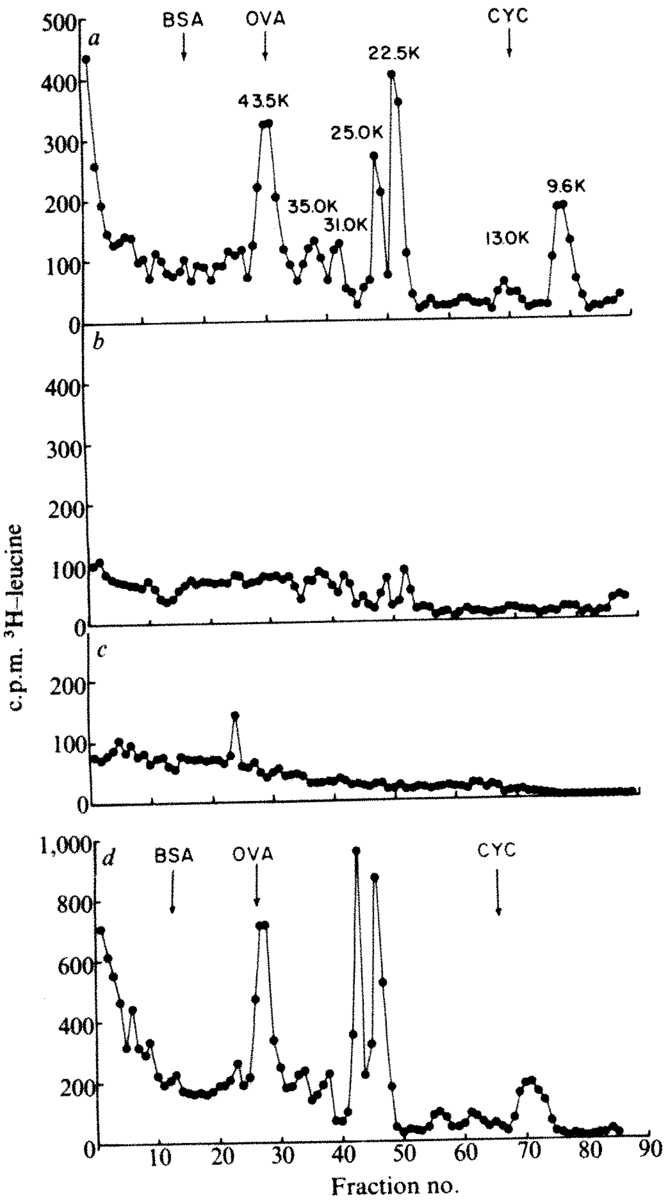

Fig. 3 Protein synthesis in wild-type and mutant mitochondria. Cells grown in roller bottles (~ 5–7 × 10^7 cells total) were incubated for 30 min with 100 μg ml^{-1} cycloheximide, and then labelled for 2 h with ^3H-leucine (20 μCi ml^{-1}; specific activity 57.4 Ci $mmol^{-1}$), followed by a 30-min chase with an excess of cold leucine (100 μg ml^{-1}). The mitochondria were purified first as described in the legend of Table 1, then loaded on top of linear sucrose gradients (1.03–1.60 M sucrose, 50 mM Tris, *p*H 7.4, 2 mM EDTA) and centrifuged at 4 °C in a Beckman SW50.1 rotor at 40,000 r.p.m. for 2 h. A narrow band of material was always observed at a density of 1.17 g cm^{-3}, and a peak of radioactivity was associated with this band. The band was removed, the material pelleted by centrifugation, dissolved at 90–100 °C in buffer containing Tris-HCl (62.5 mM, *p*H 6.8), glycerol (10% w/v), β-mercaptoethanol (5% w/v), sodium dodecyl sulphate (SDS, 2.3% w/v), and stored at –20 °C until ready for use in electrophoresis. Samples were run on 12.5% polyacrylamide slab gels (1 mm thickness) containing Tris-HCl (37.5 mM, *p*H 8.8) and SDS 0.1% w/v). The gels were stained with 0.1% Coomassie blue in 50% trichloroacetic acid for 20 min, destained in 7% acetic acid, and then cut into 1-mm sections with a gel slicer made of a battery of razor blades. Slices were incubated overnight in 0.5 ml NCS solubiliser (Amersham/Searle) before being counted in 5 ml of toluene-based scintillation fluid. Bovine serum albumin (BSA), ovalbumin (OVA) and cytochrome *c* (CYC) were run on the same gels in parallel tracks as molecular weight markers. Profiles for mitochondria from: *a*, V79 wild type; *b*, V79-G7; *c*, V79 pretreated with chloramphenicol at 100 μg ml^{-1} for 5 h before addition of label, *d*, CCL16-B2, a mutant lacking NADH-oxidase activity. Radioactivity content: *a*, 469 c.p.m. $μg^{-1}$; *b*, 196 c.p.m. $μg^{-1}$; *c*, 158 c.p.m. $μg^{-1}$; *d*, 836 c.p.m. $μg^{-1}$.

was distributed as a background or relatively high molecular weight material.

To prove that the observed pattern of protein synthesis is indeed due to mitochondrial protein synthesis, we tested its sensitivity to the specific inhibitor chloramphenicol (Fig. 3*c*). The effect of this inhibitor is very marked, although we found it necessary to pre-incubate the cells for some time with the drug before the addition of ^3H-leucine. With wild-type V79 cells we found approximately 64% inhibition of cycloheximide-resistant ^3H-leucine incorporation as well as an altered profile (Fig. 3*c*). Pretreatments longer than 5 h failed to reduce the level of incorporation further. We tentatively conclude that there is a background of chloramphenicol-resistant protein synthesis in this system (which may in fact be due to incomplete inhibition of cytoplasmic synthesis) which accounts for approximately one-third of the incorporation seen in the absence of the drug.

The abnormal pattern of mitochondrial protein synthesis in V79-G7 cells is not a general characteristic of respiration-deficient Chinese hamster fibroblasts. Figure 3*d* shows a profile corresponding to mitochondrial protein synthesis in a mutant defective in NADH-coenzyme Q reductase[9,10]. Except for a difference in specific activity there is no significant difference between this pattern and that observed for wild-type cells, and similar results were observed with yet another respiration-deficient mutant representing a third complementation group (results not shown). We conclude that the defect in mitochondrial protein synthesis in V79-G7 cells is unique.

We do not yet know whether the mutation in the V79-G7 cells is nuclear or cytoplasmic. In fact, this mutant is very similar in phenotype to a nuclear yeast mutant (strain M126) described by Colson, Labaille and Goffeau[19]. Mitochondrial DNA is known to code for a limited number of macromolecules including ribosomal RNAs, transfer RNAs and probably certain subunits of cytochrome oxidase, cytochrome *b*, and the oligomycin-sensitive ATPase (refs 13, 14, 16, 17, 20), while most if not all of the ribosomal proteins, RNA polymerase, aminoacyl-tRNA synthetases, and other protein factors involved in protein synthesis are thought to be coded for by nuclear genes and imported from the cytoplasm[21,24].

Studies are in progress aimed at (1) the fusion of enucleated, cytoplasmically marked (chloramphenicol-resistant) hamster cells with the V79-G7 cells; (2) measurement of the spontaneous reversion rate; and (3), physical characterisation of the mitochondrial DNA from the mutant. Further investigation of this mutant may allow further comparison of mitochondrial biogenesis in eukaryotic cells such as yeast and in mammalian cells, and of the contributions which the nuclear and mitochondrial genomes make to the assembly of the organelle.

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Interferon production during lymphocytic choriomeningitis virus infection of nude and normal mice

SEVERAL reports[1–6] have indicated that lymphocytic choriomeningitis virus (LCMV) infection of mice or cultured cells is not associated with interferon production. But, as was found later[7] the methods used may not have been sensitive enough to detect interferon in tissues of virus infected animals. In contrast to these earlier observations, mice acutely infected with M-P virus, a virus closely related to LCMV (ref. 8), have been reported to produce interferon[9]. We observed a more intense and prolonged interferon response to a virus inducer in nude athymic mice (Fig. 1) as others[10] have with synthetic double-stranded nucleic acids. Therefore, it seemed reasonable to study LCMV infection of nude animals carefully for interferon production in serum and infected organs. Parental strains and nude heterozygotes (*Nu*/$^+$) were studied as well as the homozygous nude (*Nu*/*Nu*) animals. The results presented here indicate that interferon is made in greater quantity and persists longer during infection of *Nu*/*Nu* than of parental and *Nu*/$^+$ mice.

Figure 1 presents results following intravenous inoculation of live Newcastle disease virus in *Nu*/*Nu*, *Nu*/$^+$ and BALB/c parental strains, as well as similar parental and nude mice of a CBA background. Results from two separate serum pools involving 10 animals each were averaged for both comparative studies. It can be seen that the parental responses are consistently lower than those of nude animals at all time points, particularly at 12 and 24 h after virus inoculation. The *Nu*/$^+$ mouse in our studies with the CBA strain resembles the nude mouse but is intermediate in its responses in the BALB/c animals. These findings resemble those reported by Yokota, *et al.*[10] with poly I : C injection of BALB/c, nude and heterozygote animals, and indicate that interferon production can occur in the absence of thymus dependent lymphocytes.

Studies with acute infection demonstrated high interferon titres in the spleen and serum in all 6-week-old animals studied 4–7 d after intracerebral (i.c.) inoculation with 100 LD$_{50}$ of LCMV, Armstrong strain (Table 1). This time period corresponds to the period of maximal T-cell activity and other immunopathological responses. In the nude animals there was an increase in spleen and serum levels of interferon, four- and threefold respectively on the seventh day after infection compared with the fourth day after infection. In contrast, at least an eightfold reduction in interferon levels was observed in the spleens and serum from *Nu*/$^+$ and parental BALB/c animals studied at similar times (Table 1). The antiviral material extracted from the spleen and serum was shown to be interferon by its stability to *p*H 2 for 1 h at 4 °C, lack of sedimentability at 100,000*g* for 2 h, its inactivation by trypsin (1 mg ml^{-1}, 37 °C 1 h), its immunospecific neutralisation on exposure to antisera to L cell interferon[12] and its lack of protection of human skin fibroblasts challenged with vesicular stomatitis virus. Furthermore, no protection was observed when the antiviral substance was applied to L cells for only a short period (1 h) before virus challenge even though it was maintained on monolayers during plaque development.

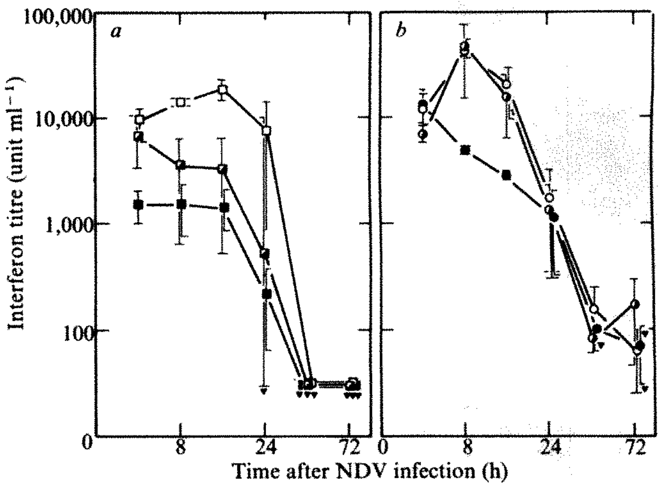

Fig. 1 Production of interferon in *Nu*/*Nu*, *Nu*/$^+$ and BALB/c or CBA parental strains. *a*, BALB/c, *Nu*/*Nu* and *Nu*/$^+$; *b*, CBA, *Nu*/*Nu* and *Nu*/$^+$. Each point represents the mean of two experiments in which serum from 10 animals was pooled for each experimental observation. The serum was collected at the indicated times after inoculation of 20-g mice with 1 × 10^8 plaque-forming units of live Newcastle disease virus (NDV) intravenously. Interferon titres represent the reciprocal of the dilution of serum giving 50% inhibition of vesicular stomatitis virus plaque formation in the L$_{929}$ monolayers growing in 60-mm plastic plates as described elsewhere[11]. The mean and range of replicates is shown by the bracketed bar. Symbols: in *a*—■, homozygote BALB/c parent (BALB/c); ◪, BALB/c nude heterozygote (*Nu*/$^+$); □, nude homozygote (*Nu*/*Nu*). In (*b*), ●, CBA parent (CBA); ◑, CBA nude heterozygote (*Nu*/$^+$); ○, nude homozygote (*Nu*/*Nu*). In all strains, interferon titres in the serum at zero time were less than 20 units per ml.

Interferon levels in the liver and kidney were similar in all animals 4 d after LCMV inoculation, while at the seventh day post-infection, nude mice showed higher levels of interferon when compared to *Nu*/$^+$ and parental mice. Despite high virus titres and in contrast to certain other murine viral encephalidites, interferon titres in the brain were low and indistinguishable in all three types of animal. It is of interest that there were higher virus titres in spleen, liver and kidney tissues of nude mice 7 d after infection as compared with *Nu*/$^+$ and BALB/c animals. The nude animals also survived acute LCM infection in contrast to the parental and *Nu*/$^+$ animals.

These studies demonstrate that mice acutely infected with Armstrong strain of LCMV make interferon and that acutely infected T cell-deficient mice make greater amounts of interferon, particularly at the sixth and seventh days after initiation of infection, concomitant with their survival. It has been shown by various depletion and reconstitution studies that T-derived lymphocytes sensitised to LCMV (refs 14–16) as well as antibody to LCMV and complement[17] are associated with tissue injury in acute or chronic LCMV infections[13]. The transfer of sensitised spleen cells[18] is associated with significant decreases in viral

Table 1 Interferon and virus titres after LCMV infection of BALB/c, *Nu/Nu* and *Nu/*$^+$ mice

		Interferon and virus titres		
Organ	Day after infection	*Nu/Nu*	*Nu/*$^+$	BALB/c
Spleen	4	2,000*	2,580	1,400
	6	4,100	130	100
	7	8,250	271	50
		(5.9×10^6)†	(8.8×10^4)	(1.24×10^4)
Liver	4	250	456	70
	6	1,200	1,100	800
	7	170	70	70
		(5.8×10^4)	(2×10^3)	(2×10^3)
Kidney	4	200	220	24
	6	215	180	160
	7	120	20	15
		(2.4×10^5)	(8.4×10^3)	(10^3)
Brain	4	20u	10	20
	6	35u	75	25
	7	20u	35	15
		(7.3×10^7)	(1.5×10^7)	(5.8×10^7)
Serum	4	1,400	1,650	460
	6	2,150	100	30
	7	4,150	205	60
Mortality	10	0%(0/10)‡	100%(10/10)	100%(10/10)

Acute infection was induced at 6 weeks of age with 100 LD$_{50}$ of LCMV i.c.

*Units of antiviral activity per gram of tissue or ml of serum as determined by titration of a pool obtained from five mice.

†Figures in parentheses represent virus titre in PFU per gram of organ as measured by BHK cell monolayers (results are an average of five individual single organ titrations).

‡Animals surviving/animals challenged. No homozygote nude mouse died of acute infection. With both heterozygotes and parental strains, approximately 50% died by day 7, 80–90% by day 8, and all by day 10.

titres and transfer of sensitised spleen cells[18] or depletion of complement[17] is associated with enhanced survival, although the bulk of evidence indicates that T-derived lymphocytes have a major role in survival and/or immunopathological injury. The observations reported here suggest that high tissue interferon levels in the nudes may perhaps also have a protective role in this virus infection. With recent observations[19–24] of an immunoregulatory action of interferon, it is possible that interferon may directly modulate some of the altered immunological responses observed in LCMV infected nude compared with controls. Unfortunately, our findings provide no direct evidence for this possibility. In addition, we found that BALB/c and *Nu/*$^+$ mice dying of acute LCMV infection have high titres of virus in their brain tissues together with low levels of interferon and abundant tissue injury. The latter results contrast with the high titres of both interferon and virus found in other tissues and modify the alternative interpretation that interferon production is just a concomitant of high concentrations of LCMV.

The antiviral substance we have observed in this report and have identified as interferon should be differentiated from the inhibitor of virus replication observed by Veltri and Kirk[25] in animals acutely infected with LCMV. Their inhibitor was present in higher titre in the brain than in the spleen and was inactivated by *p*H 2 exposure. It also demonstrated activity on human cells. Furthermore, the action of their inhibitor did not persist in the treated cells and was eliminated with a media change whereas ours was not.

In summary, our studies have shown that nude mice have higher titres of interferon in their spleens and serum during LCM infection concombinant with higher virus titres in various tissues and their survival from this infection. The significance of increased interferon production in the survival of these animals remains to be determined. Their survival is undoubtedly favoured by their inability to mount T lymphocyte mediated immunopathological responses.

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Immunological tolerance and tumour allografts in the brain

THE brain has been considered to be an immunologically privileged site with regard to tissue transplantation. In addition, a loss of 'tumour surveillance' due to a weakening of the immune system in the brain has been postulated. The data on the brain as a privileged site are not unequivocal, however. The evidence for and against this thesis has been discussed by Woodruff[1] and Lance[2]. Barker and Billingham[3] also speculated that "reports that the brain can prevent implanted homografts from inciting sensitivity because it lacks a lymphatic drainage, thus having no afferent pathway of the immunological reflex, are more equivocal". The need for a critical re-evaluation of the brain as a privileged site has been stressed. We report here a study of the possible action of immunological tolerance in the brain and of the sensitivity of normal rats and tolerant rats to inoculation of allogeneic tumour cells into the brain. We show that transplantation tolerance is involved even in the brain; thus demonstrating the presence of specific immunity following inoculation of tumour cells into the brain. This finding suggests that previously held views of the brain as a privileged site may not be entirely valid and that specific immune processes are accomplished in the central nervous system.

AVN strain rats were used as recipients. The donors were Lewis strain rats, differing from the AVN recipients in H-1 plus non-H-1 antigens. Immunological tolerance was induced in newborn AVN recipients by the intravenous injection of $40–45\times10^6$ bone marrow cells from Lewis donors. Skin grafts from Lewis rats were transplanted 2 months after birth. Tumour cells which were inoculated into the brains were obtained from the culture of a virogenic sarcoma RSL. The RSL sarcoma was induced in a newborn Lewis rat by injection of the Schmidt–Ruppin strain of Rous sarcoma virus[4]. 5×10^3–5×10^6 tumour cells in a volume of 0.05 ml of Eagle's medium were injected with a microsyringe into the occipital lobe in the right hemisphere 1.5– [illegible] in depth of 4-month-old tolerant and untreated control AVN recipients and syngeneic Lewis recipients. After tumour cell inoculation, the animals were inspected daily and the effect of tumour growth was evaluated by death of the animals. Some animals were taken for histological examination.

The results show that the action of transplantation tolerance extends even to the brain. While untreated animals were resistant to tumour cell inoculum, except for the largest dose of 5×10^6 cells, all of the tolerant animals, like the syngeneic recipients, died after even the smallest dose of 5×10^3 cells (Table 1). Some of the non-tolerant animals died within 5–11 d of allotransplantation of the largest dose of tumour cells, whereas deaths in tolerant and syngeneic recipients of the smaller doses occurred at a slower rate (Table 2). The resistance of untreated animals to the inoculated tumour allografts was immunologically specific, as it was abolished by the induction of allogeneic tolerance. When large doses of tumour cells were inoculated into the brain some non-tolerant recipients died. RSL tumour cells injected into the muscle of the hind feet of adult AVN rats in amounts up to 10^7 cells did not induce distinct tumour growth. In 2-month-old rats the dose of 10^7 cells gave rise to tumours, but these regressed within 16 d and no animals died.

Table 1 Mortality of allogeneic normal and tolerant rats, and of syngeneic rats, following inoculation of tumour cells into the brain

No. of injected tumour cells from Lewis rats	Allogeneic AVN recipients Untreated	Allogeneic AVN recipients Tolerant	Syngeneic Lewis recipients Untreated
5×10^3	0/10*	5/5	9/9
5×10^4	1/10	8/8	NT†
5×10^5	0/10	NT	NT
10^6	0/20	10/10	5/5
5×10^6	14/24	NT	NT

*Numerator, number of dead animals; denominator, total number of animals.
†NT, Not tested.

Thus, tumour tissue allografts grew relatively better in the brain than in other sites; this seems to agree with the findings of Murphy and Sturm[5], who investigated the survival of tumour xenografts. Our observations that tumour allografts in the brain are more fatal than those in limb muscle may be, however, due to the fact that the tumour growing in the brain kills the animal before a sufficiently intense immune reaction develops. That is, the comparison may not be quite fair as the anatomical difference may simulate an immunological privilege.

Histological findings in normal animals showed the presence of lymphocytes in the brain tissue, and these cells could have participated in the immune reaction evoked by injected tumour cells. In the proximity of tumour cells, infiltrates of lymphocytes were observed in the perivascular spaces. The immune reactions in the brain were demonstrated in the experiments of Šterzl and Lodin[6] in which the administration of antigen to the brain led to marked antibody formation by the immigrating cells localised in the brain. Thus, it seems that the vascularisation of the brain is sufficient to mediate the afferent and efferent arcs of the immunological reaction. Consistent with this notion are the speculations of Tilney and Gowans[7] who posed the question "if kidneys can sensitise their hosts by way of the blood, why cannot fully vascularised grafts in immunologically privileged sites do the same?" The vascularisation itself has an important role in the survival of the tissue implants in the brain[5,8]. But it is difficult to explain the long-term survival of allogeneic normal or tumour tissues, as observed by some investigators (see refs 1 and 2 for reviews). The avascularity of the implants might be the cause. Although the concept of the brain as an immunologically privileged site is still, to some extent, in dispute, the data reported here clearly show that after tumour inoculation the basic immunological reactions are carried out in the brain.

Table 2 The mortality range of allogeneic tolerant and syngeneic rats following inoculation of tumour cells into the brain

Recipients	No. of inoculated tumour cells 5×10^3	5×10^4	10^6
	Deaths on days		
Allogeneic tolerant	17–21	12–16	6–10
Syngeneic untreated	17–21	NT*	6–9

*NT, not tested.

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Macrophages isolated from regressing Moloney sarcomas are more cytotoxic than those recovered from progressing sarcomas

THERE is increasing evidence that many tumours contain appreciable numbers of macrophages[1–3], and that these inflammatory cells can be cytotoxic for various neoplastic cells *in vitro*[4,5]. Thus at first it seems paradoxical that many tumours are progressive and lethal in spite of a high content of macrophages. But to kill a malignant cell, these inflammatory cells must be brought to an ill-defined state of 'activation'[6,7]. It is therefore conceivable that the macrophages in progressing tumours are less activated, and thus less cytotoxic, than those in spontaneously regressing neoplasms. To test this hypothesis we isolated macrophages from either regressing or progressing Moloney sarcomas and tested them *in vitro* for their capacity to release chromium-51 (^{51}Cr) from prelabelled target cells. The former were usually significantly more cytotoxic than the latter.

At the times of collection (7–13 d after inoculation) regressing tumours (regressors) were 0.3–0.5 cm in diameter, firm and somewhat difficult to distinguish from adjacent normal skeletal muscle. By contrast, progressing tumours (progressors) were 1–2 cm in diameter, soft and friable and contained no grossly identifiable muscle. There were small foci of recent haemorrhage in the centres of the larger tumours. Progressors, left to run their course, kill the host after metastasising to the lung[8,9], while regressors are rejected completely, usually by the fourth week after inoculation[3,8]. Regression is attended by an extensive mononuclear inflammatory response[8,10], shown in tumour disaggregation studies to consist principally of T lymphocytes and macrophages[3]. Both these inflammatory cell types are also found associated with progressors, but unlike regressing sarcomas, inflammatory cells do not penetrate beyond the peripheries of progressing neoplasms.

Tumours were disaggregated as before[11], and macrophage monolayers were established from the resultant cell suspensions[12]. Macrophages in monolayers were counted in Giemsa-stained monolayers after exposure to zymosan particles. As measured by release of ^{51}Cr (details in legend for Table 1), at a density of 1,500 mm^{-2}, macrophages recovered from regressing Moloney sarcomas killed both the P815 mastocytoma and SV40-transformed 3T3 target cells (Table 1), albeit with different efficiencies (probably reflecting differences in target cell susceptibility). By contrast, neither target cell was affected by macrophages collected from progressors. Similar results at this macrophage density have been obtained in three experiments.

The density of macrophages in monolayers is of critical importance in demonstrating their cytotoxicity. Several groups have shown, for example, that maximal killing by these cells is not obtained unless monolayers are confluent, or nearly so. Doe and Henson (unpublished results) have investigated this aspect of macrophage-mediated cytotoxicity in detail, using the basic assay system described here. When thioglycollate broth-induced peritoneal macrophages were activated by bacterial lipopolysaccharide, Doe and Hensen obtained no measurable cytotoxicity at monolayer densities of <3,000 mm^{-2}, while maximal effects (that is, >80% specific ^{51}Cr release) were obtained at macrophage densities of 5,000 mm^{-2} or more. In view of these findings it is clear that the experiments we have described, where monolayers were 50–60% confluent, did not distinguish whether progressor macrophages were absolutely unable to cause ^{51}Cr release or, rather, that they were simply diminished in their cytotoxic capabilities compared with regressor macrophages. We therefore addressed this point by establishing, for regressors and progressors, a series of monolayers of increasing macrophage density (details in legend for Fig. 1). Similar results were obtained in approximately 80% of all such experiments (postinoculation days 7–13), suggesting that progressor macrophages can kill, but that their relative cytotoxicity is much less than that of macrophages recovered from regressors. There was some variation, however, for occasionally (3 per 14 experiments) progressor macrophages gave a level of killing nearly as high as or, in one case, higher than that obtained using regressor macrophages. In contrast to the small amount of killing usually obtained with progressor macrophages, such results were obtained sporadically and were not reproducible. The cause of such variation may be technical or stem from some undefined variable associated with the tumour system.

Table 1 Cytotoxicity of macrophages recovered from regressors or progressors

Target cell	Source of macrophages	c.p.m. in supernates of triplicate cultures (mean ± s.d.)	% Corrected ^{51}Cr release
P815 mastocytoma	None	2,152±11	—
	Progressor	2,163±65	0.2
	Regressor	4,127±35	32.0*
SV40-transformed 3T3 cell	None	1,808±14	—
	Progressor	1,798±56	−0.2
	Regressor	2,247±92	10.0*

Regressing or progressing Moloney sarcomas were induced in 6–8-week-old, male, BALB/c mice by intramuscular injection of either 5×10^3 or 10^6 cultured MSC cells, respectively[3,8]. Tumours collected 13 d later were disaggregated using a stirred mixture of trypsin, collagenase (each 0.1 mg ml^{-1}) and DNAse (0.025 mg ml^{-1}) (ref. 11). Macrophages in resultant cell suspensions were plated in 16-mm diameter, flat bottomed wells of plastic tissue culture trays (Linbro). Monolayers (50–60% confluent) containing 3×10^5 cells per well (1,500 per mm^2) (determined by cell counting using an inverted microscope equipped with a gridded ocular of known area) were obtained by brief exposure of each well twice to 2.5×10^6 cells from the crude suspension in 0.5 ml HEPES-buffered (15 mM) minimum essential tissue culture medium (HMEM) containing 10% foetal bovine serum (FBS). Five minutes after each seeding plates were agitated and all non-adherent cells were washed away after a total of 10 min. Ninety per cent of the adherent cells from both regressors and progressors were macrophages, as determined by microscopic examination of representative, Giemsa-stained monolayers treated and untreated with zymosan particles. The principal contaminants were tumour cells and polymorphonuclear leukocytes. Target cells antigenically unrelated to MSC cells, and therefore insusceptible to killing by the rare T lymphocyte which may have contaminated effector cell populations, were labelled for 1 h in HMEM+10% FBS containing sodium ^{51}chromate (250 μCi ml^{-1}, Amersham/Searle, Arlington Heights, Illinois; specific activity 200 mCi mg^{-1}). After washing, targets were allowed to stand at 37 °C for 2 h (with agitation at 30-min intervals), counted, resuspended in HMEM+30% FBS and added to wells (10^5 each; macrophage-to-target cell ratio 3:1). After incubation at 37 °C for 17 h, half of each culture supernate, collected by centrifugation, was assayed for radioactivity in an automatic gamma scintillation spectrometer. Percentage corrected ^{51}Cr release was computed using the formula ((experimental ^{51}Cr release − spontaneous ^{51}Cr release) ÷ (freeze-thaw ^{51}Cr release − spontaneous ^{51}Cr release)) × 100. Negative values indicate that release in experimental cultures was less than spontaneous release. Rates of spontaneous ^{51}Cr loss by P815 and SV40-transformed 3T3 cells were 1.5% and 1.7% per h, respectively.

*As determined by Student's *t* test, the differences in cytotoxicity between regressor and progressor macrophages are significant at the <0.01 (SV40 3T3) and <0.001 (P815) levels.

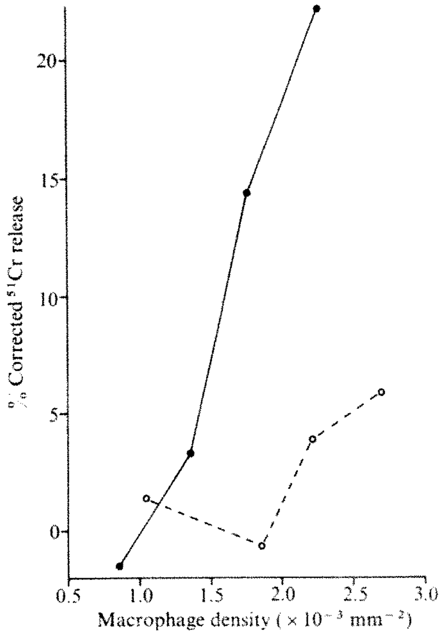

Fig. 1 Cell suspensions were prepared from either regressing (●) or progressing (○) Moloney sarcomas (postinoculation day 8) and plated as described under Table 1 and in ref. 12. Numbers of macrophages were increased by repeated exposures of Linbro wells (200 mm^2) to the crude cell suspension. The lowest density was obtained by a single exposure of 5 min, the second by one exposure of 10-min, the third by two 10-min periods, and the highest by three 10 min incubations. The wells were washed after each exposure. Specific ^{51}Cr release was assayed as described under Table 1.

Thus it seems that macrophages recovered from regressing Moloney sarcomas generally are more cytotoxic than those obtained from progressing neoplasms of the same type. There is precedent for this finding in the work of

Fidler[6] and G. Miller and J. Feldman (personal communication). In both cases peritoneal macrophages induced by injection of thioglycollate broth were cytotoxic if the animal from which they came had been pre-immunised against the tumour target cell used, but were unable to kill or were greatly diminished in this capacity, if collected from an animal which carried a progressing tumour induced by the same target cell. Fidler's work further suggested that the observed inability to kill was not an innate deficiency of the macrophage, but was due to lack of macrophage activation by soluble lymphocyte mediators. One possible explanation for the diminished cytotoxicity exhibited by progressor macrophages therefore is inadequate activation of these cells by intratumoral lymphocytes. Indirect support for this hypothesis is found in the recent demonstration that, in at least one regard (direct mediation of cytotoxicity), T lymphocytes recovered from large progressing Moloney sarcomas were functionally inactive[13]. Direct testing of the macrophage activating potential of progressor T lymphocytes is in progress.

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Aspartic acid racemisation in the human lens during ageing and in cataract formation

D-ASPARTIC acid has been shown to accumulate with age in human tooth enamel[1] and dentine[2] at a rate of about 0.1% yr^{-1}. We have predicted that racemisation should take place in any metabolically stable protein in long-lived mammals and that, as a consequence of racemisation, these proteins will have altered conformations which would probably produce changes in their biological activities or chemical properties[3]. We have extended these studies to soft tissue proteins, in particular those in the human lens. The proteins in the central portion of the lens are among the most stable in the human body[4]. Numerous changes in the properties of the lens proteins occur with age and cataract formation—denaturation[5], increasing pigmentation[6], cross linking[5,6] insolubility[6–9], and fluorescence[10]. Pirie[11] has suggested that' physicochemical processes may be responsible for these changes. We report here the results of D/L enantiomeric analyses of normal human lenses and cataracts: aspartic acid racemisation was seen during ageing and cataract formation.

Human lenses were obtained from Eye Bank collections or after surgical extraction at the Duke University Medical Center. Cataracts were graded according to the colour classification of Pirie[6]: group I, pale yellow lens; group II, pale cortex with visible nucleus; group III, pale cortex with hazel brown nucleus; group IV, pale cortex with deep brown nucleus. The central 15% by weight of each lens was removed by the method of Spector *et al.*[10]; these samples will be referred to as the central nuclei. A freshly extracted cataract lens from a 76-yr-old individual was sectioned as above. Then two additional samples were taken from the remainder of the lens, one representing the inner cortex and a second from the peripheral cortex. All lens samples were stored frozen.

The samples were processed using procedures described elsewhere[12,13]. Measurements of D/L aspartic acid ratios were performed on a Beckman-Spinco Model 118 automatic amino acid analyser, using the diastereomeric dipeptide technique[14]. The D/L enantiomeric ratios for glutamic acid, proline, alanine, phenylalanine, and leucine were determined by gas chromatography. The *N*-trifluoroacetyl-L-prolyl peptide methyl esters were synthesised, and separation was performed by gas chromatography (Hewlett-Packard Model 5711A with flame ionisation detector; 20-foot glass column packed with 8% SP 2250 coated on Chromosorb W-AW-DMCS solid support[15]).

The D/L aspartic acid analyses for the central nucleus samples of 17 normal human lenses are plotted in Fig. 1. The line in Fig. 1 represents a least squares fit to the normal lens data, and the equation for this line is

$$\ln\left(\frac{1+\text{D/L}}{1-\text{D/L}}\right) = (2.50\,(\pm 0.29)\times 10^{-3}\text{yr}^{-1})(t+0.112) \quad (1)$$

where t is the age of the individual. The data are presented in the form of the reversible first order rate equation[16], rather than the irreversible equation used for the enamel and dentine results[1,2], because D-aspartic acid is present in sufficient concentrations in the lenses to make the use of the irreversible first order rate equation invalid. The slope in equation (1) corresponds to $2\,k_{\text{asp}}$. Thus, in the lens nucleus, $k_{\text{asp}} = 1.25\times 10^{-3}\,\text{yr}^{-1}$. The correlation coefficient (r) for the lens results is 0.912.

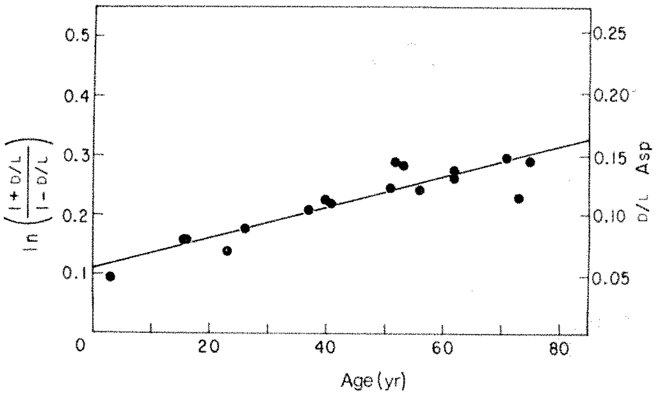

Fig. 1 Analysis of D/L-aspartic acid contents of central nucleus samples of 17 normal human lenses.

The y-intercept, 0.112, is equivalent to a D/L ratio of 0.056. This ratio is higher than the enamel[1] (0.033) and dentine[2] (0.017) $t = 0$ values. A possible explanation for this difference is that the primary structures of proteins may influence the amount of acid-catalysed racemisation which occurs during the hydrolysis step[14].

Since the proteins in the central nucleus are thought to be 'older' than the proteins in the peripheral regions of the lens, the D/L-aspartic acid ratios should be higher in the nucleus than in the cortex in samples from the same lens. The D/L ratios obtained for the nucleus, inner cortex, and peripheral cortex of the 76-yr-old cataract are 0.171, 0.167, and 0.047, respectively. Only the peripheral cortex shows evidence of recent protein synthesis. The peripheral cortex result is identical to the D/L ratio from the 3-yr-old lens nucleus, that is, 0.047, and may provide a closer approximation to the $t = 0$ value for human lens proteins than the extrapolated value of 0.056.

Our studies of racemisation rates in teeth[1,2] led us to propose that D/L-aspartic acid analyses can be used as a biochronological tool to estimate the ages of long-lived mammals. Racemisation in the lens nucleus can be used in a similar manner. There are several advantages to working with lenses rather than teeth for mammalian age determinations. (1) Temperature is probably higher and more constant. (2) Teeth of certain mammals, such as cetaceans, have a rather complex pattern of development in which dentine growth layers are laid down well into adulthood[17]. (3) Normal tooth attrition makes sampling from old animals difficult. (4) The faster k_{asp} in lenses permits young ages to be calculated more accurately.

The extent of aspartic acid racemisation in the central nuclei of 13 group I and II cataracts was determined, and a least squares fit of these data yielded $k_{asp} = 1.29 \times 10^{-3}$ yr^{-1} ($r = 0.875$), which is indistinguishable from the rate in normal lenses. This is particularly interesting since the cataracts included subcapsular, nuclear, and cortical types associated with different aetiologies or disease states (trauma, diabetes, hypertension, glaucoma, Parkinson's disease and so on). Thus, despite the development of these types of cataracts, the racemisation rate is identical to that observed in the lens nucleus during the normal ageing process.

The brunnescent group IV cataracts listed in Table 1, on the other hand, have a much greater degree of aspartic acid racemisation than we have observed in any stable human proteins. These cataracts have D/L-Asp ratios 58–97% higher than either the normal lenses or group I and II cataracts of similar ages. One yellow cataract of unknown age also had a very high D/L ratio of 0.265, which is equivalent to the brunnescent lens values, while a group III cataract (76 yr. D/L Asp = 0.171) falls within the range of the yellow cataracts. Therefore, enhanced aspartic acid racemisation is strongly but not invariably associated with the development of brown pigment during cataract maturation.

Table 1 Extent of aspartic acid racemisation in brunnescent cataracts

	D/L-aspartic acid	
Age (yr)	Expected*	Observed
57	0.127	0.203
76	0.150	0.296
77	0.151	0.268
80	0.155	0.283
89	0.166	0.262

*The expected ratios were calculated from equation (1) using the age of each individual.

Gas chromatographic analyses were performed on two normal (3- and 75-yr-old), seven yellow, and three group IV lenses to determine the D/L enantiomeric ratios for alanine, glutamic acid, proline, phenylalanine and leucine. No racemisation of these amino acids was observed in any of the lenses. (The expected ratios[18] are at the limits of detection by this technique, however). Thus, only aspartyl residues are racemising to a measurable extent in the nuclear proteins of these lenses, which is the expected result[18]. Also, our results suggest that only aspartic acid exhibits an accelerated rate of racemisation in the brown cataract lenses.

If aspartic acid racemisation has a role in biological processes, including ageing, the reaction must occur in a variety of tissues. D-aspartyl residues have been shown to accumulate in normal lens nuclear proteins at a rate of 1.25×10^{-3} yr^{-1} or 0.14% per year, and therefore aspartic acid racemisation should take place with age in any stable soft tissue protein in long-lived mammals.

The rate constant for the lens nucleus is about 1.5 times the rate observed in tooth enamel and dentine. Although these rate constants fall within two s.d. of each other, several variables could be contributing to the difference in rates. The most obvious explanation is that the temperature of the lens is probably more constant and slightly higher than that of teeth, leading to a more rapid racemisation rate. Alternatively, the different protein environments in calcified and non-calcified tissue could explain the differences. The accelerated aspartic acid racemisation rate associated with brunnescent cataracts cannot be explained by the above factors. Among the possible mechanisms which could give rise to an accelerated rate during cataract maturation are change in the pK_a of the β-carboxyl group due to alteration in the microenvironment within the protein[19] and chelation of the β-carboxyl group by trace metals[18].

We have suggested that D-aspartyl residues should alter the conformation of a protein and that these effects will become more pronounced as the D-enantiomer accumulates with age[3]. A number of studies indicate that structural changes occur in lens proteins with age and in cataracts. The proportion of water-insoluble protein increases with age[8,9], and the increase is even more marked in cataracts[6,8]. The water-insoluble residue can be refractionated with concentrated urea. The urea-insoluble portion is greater in cataracts[6] and occurs primarily in the lens nucleus[7]. The proteins in cataractous lenses have an increased number of exposed thiol groups and a greater susceptibility to tryptic digestion, which suggests that conformational changes are taking place during cataractogenesis[5]. Cataracts show evidence of more disulphide[20,21] and non-disulphide[7] covalent cross links than do normal lenses, and the cross links increase during cataract maturation. It is possible that aspartic acid racemisation may actually be induced by structural changes in the lens proteins initiated by other factor(s). But, the above observations are also consistent with the predicted consequences of the racemisation of aspartyl residues.

We do not know whether the accelerated rate of racemisation of aspartic acid contributes to the development of brunnescent cataracts or whether during cataract formation the aspartyl residues become more susceptible to racemisation. Since the percentage of urea-insoluble protein increases more than fourfold between yellow and group IV cataracts[22], protein alteration seems to coincide with the increase in racemisation rate.

Regardless of the actual mechanism responsible for the accelerated rate of aspartic acid racemisation in cataract maturation, this chemical alteration provides important information on processes taking place in the human lens during the development of a pathological condition. Elucidation of this mechanism may provide new insights into the process of cataract maturation.

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A role of selenium against methylmercury toxicity

SINCE Parizek *et al.*[1] reported that administration of selenite clearly decreased toxicity of mercuric chloride, there have been many studies on the mechanisms responsible for the interrelationship between selenium and inorganic or organic mercury. Almost all have shown that inorganic selenium, especially selenite, prevents growth inhibition as well as mortality and neurotoxicity due to methylmercury given simultaneously in the diet of Japanese quail[2-4] and rats[5-8]. Protective effects of selenium against methylmercury toxicity do not involve mercury absorption through intestines[9] or excretion in the urine and faeces[6]. Selenite does not increase the rate of breakdown of methylmercury[7], and thionein does not have a significant role in the detoxification of methylmercury[3], but presumably, the form of methylmercury is modified in some way by selenium. We present here evidence that selenite can release methylmercury from its linkage with proteins and thereby influence its tissue distribution.

The association constants of methylmercury for sulphur ligands of the sulphydryl group of proteins are extremely large[10]. But methylmercury in animal tissues can be separated from Hg–S linkage by hydrochloric acid (1 M or 2 M) and extracted with benzene, then analysed, using a gas chromatograph with an electron capture detector[11]. Methylmercury extracted with benzene in acid conditions is defined as total methylmercury, methylmercury extracted in neutral conditions as free methylmercury and the difference between them as bound methylmercury.

Selenium compounds in phosphate buffer (*p*H 7.4) were added to methylmercury and defibrinated human blood and methylmercury was extracted with benzene after 2 h of incubation. Selenite showed a surprisingly high ability to release methylmercury from blood proteins and the effect of selenate is one-tenth that of selenite (Fig. 1). Na_2SO_4, Na_2SO_3, $Na_2H_2TeO_6$ and Na_2TeO_3 were not effective in inducing release of methylmercury.

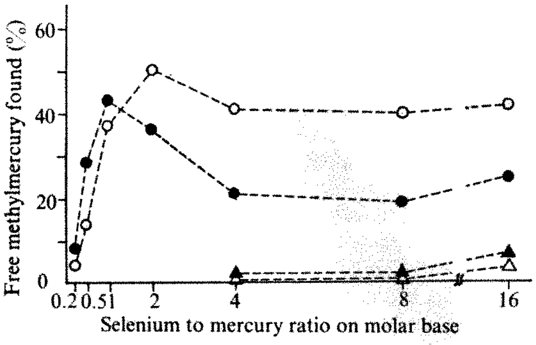

Fig. 1 Release of methylmercury from human blood by Na_2SeO_3 or Na_2SeO_4. A mixture of 0.5 ml of defibrinated blood, 5 nmol methylmercury chloride and selenite or selenate in 5 ml of phosphate buffer (*p*H 7.4) was incubated for 2 h and free methylmercury was extracted in 2 ml of benzene. The benzene layer was analysed by a gas chromatograph with electron capture detector of ^{63}Ni in the following conditions: 3 mm × 1 m glass column of Thermon 1000 on Chromosorb W at 80 ml min^{-1} of N_2 flow rate and 180 °C. ○, Na_2SeO_3 at 37 °C incubation temperature; ●, at 20 °C; △, Na_2SeO_4 at 37 °C and ▲, at 20 C°.

Addition of selenite and blood was effective in releasing methylmercury bound to bovine serum albumin, human serum, cysteine and glutathione. Addition of serum had no effect, but red blood cells effectively induced methylmercury release and did not lose effectiveness even if haemolysed by water or washed by benzene; however, they lost this ability when coagulated by heating or denatured (for example by alcohol).

Red blood cells and liver, kidney and brain homogenates from rats (Fig. 2) and mice, and muscle homogenates of raw tuna were also able to release methylmercury in conjunction with selenite. The amounts of free methylmercury released by active agents were proportionate to incubation time, amounts of biological sample, and incubation temperature. The larger the sample size and the higher the temperature, the sooner the maximum point tended to be reached. This occurred in both homogeneous and heterogeneous systems, and also in all cell fractions of mitochondria, microsome and supernatant fractions at 100,000*g*. The optimal *p*H was about 6.5 in conditions detailed in Fig. 1, with different phosphate buffers. The ratio of Se to Hg in the benzene layer was not always constant. The value of methylmercury at molar base was

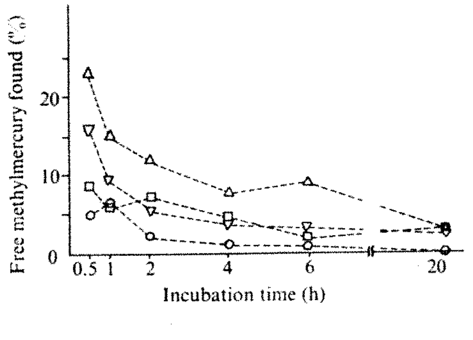

Fig. 2 Release of methylmercury from albumin by tissue homogenates and Na_2SeO_3. Methylmercury chloride (50 nmol) and 2 ml of 1% egg albumin were mixed and 50 nmol of sodium selenite and 1 ml of rat tissue or blood homogenate (each homogenate composed of one third tissue or blood and two thirds phosphate buffer) were added. Enough phosphate buffer was added to bring the total volume to 50 ml, and the mixture was incubated at 37 °C. Free methylmercury was separated from 5-ml aliquots with 2 ml benzene. □, Liver; ▽, kidney; △, brain, and ○, blood.

Table 1 The effects of sodium selenite on the *in vivo* release and tissue distribution of methylmercury

Group	No. of mice	Methylmercury content Blood TM (p.p.m.)	FM(%)	Liver TM (p.p.m.)	FM(%)	Kidney TM (p.p.m.)	FM(%)	Brain TM (p.p.m.)	FM(%)
I, Hg (control)	17	1.13±0.27	<0.1	4.04±1.49	<0.1	10.5±1.94	<0.1	1.53±0.40	<0.1
II, Hg+Se (i.p.)	17	1.25±0.28	0.14~1.5 (0.43)	3.66±0.77	0.22~2.3 (0.50)	12.4±2.31*	0.37~0.93 (0.59)	1.24±0.25*	<0.1
III, Hg+Se (i.v.)	16	0.90±0.36	0.30~45 (9.1)	3.81±1.10	0.20~7.8 (1.6)	4.45±1.34**	1.1~30 (5.1)	1.80±0.47	<0.1

TM, total methylmercury, and FM, free methylmercury. Values in FM(%) columns are the extreme ranges; figures in parentheses show the median. Group I received 100 μg of methylmercury chloride s.c. and, were killed after 1 week. Group II received 69 μg of sodium selenite i.p., 15 h before killing, which occured 1 week after methylmercury. Group III received 69 μg of selenite i.v., 30 min before killing, which occurred 1 week after methylmercury.

$^*P = 0.05$.

$^{**}P = 0.01$.

two or more times higher than that of Se estimated by flameless atomic absorption. Moreover, methylmercury from the benzene phase could be removed by cysteine solution, but Se remained largely (about 80%) in the benzene layer. This suggests that selenium may not have been associated with methylmercury. Selenite had a similar effect on methylmercury in the organs of mice treated with methylmercury *in vivo*. There was no evidence to indicate that methylmercury added was changed to dimethylmercury or to inorganic mercury.

In the *in vivo* experiments, dd-strain female mice weighing 25 g were given 100 μg methylmercury subcutaneously. About 1 week later sodium selenite, 69 μg was injected intravenously (i.v.) into the tail vein or intraperitoneally. After the selenite injection, blood samples were collected by heart puncture, and livers (detailed in Table 1), kidneys and brains removed. Blood and tissue homogenates were extracted by benzene both in the absence and presence of hydrochloric acid. After i.v. injection of selenite, free methylmercury was found in blood, liver and kidney but none in brain (Table 1). Total methylmercury content in kidney was also clearly decreased by selenite, but there were no changes in other organs.

It seems reasonable to conclude that the shift in the tissue distribution of methylmercury is a natural consequence of the liberation of methylmercury from sulphydryl bonds.

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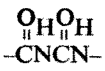
Intermolecular association and geometrical shape as two factors controlling chemical reactions

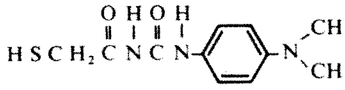
BIOLOGICAL phenomena are highly specific[1–4] and much effort has been devoted to establishing the relationship between biological activity and molecular shape. We report here the sharp dependence of selectivity in the oxidation of a pair of thiols on their structures or on environment, the mode of each dependence of selectivity being controlled dominantly by their tendency towards intermolecular association.

Two types of model systems were used. One pair of reactants can associate through intermolecular hydrogen bonding (associating system, system A) and the other pair cannot (non-associating system, system N). The model compounds (I and II) for system A are composed of three regions—the reaction site (SH group) where oxidation takes place, the binding site

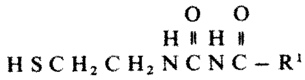


$$-\overset{O}{\overset{\|}{C}}\overset{H}{N}\overset{O}{\overset{\|}{C}}\overset{H}{N}-$$

an acylurea bond that can participate in intermolecular hydrogen bonding[5], and the recognition site ($-C_6H_4N(CH_3)_2$ or R^1) that may further participate in the discrimination. A pair of simple thiols, ethyl thioglycolate (III)



I



$$HSCH_2CH_2\overset{H}{N}\overset{O}{\overset{\|}{C}}\overset{H}{N}\overset{O}{\overset{\|}{C}}-R^1$$

II

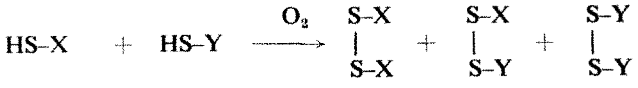
$HSCH_2CO_2C_2H_5$

III

$HS{-}R^2$

IV

and alkanethiol (IV, $R^2 = (CH_2)_kCH(CH_3)_2$), were used as the model compounds for system N. A 1:1 mixture of I and II (or of III and IV), on treatment with oxygen, gave one unsymmetrical and two symmetrical disulphides. The selectivity factor (R) in this oxidation (an indication of recognition of II by I—or of IV by III), is represented by the ratio ($V/VI = R$) of unsymmetrical disulphide (V) to symmetrical disulphide (VI) obtained from I or III.



I or III   II or IV   VI   V

Oxidation of a mixture of methanethiol and ethanethiol is known to yield the three disulphides in a 1:2:1 ratio[6]. This was also the case with oxidation of system N in 80% aqueous acetonitrile at 35 °C: the product ratio (R) was 2.1 in two cases ($k = 1$ and 2, Fig. 1). In contrast, quite different results were obtained with system A. When a series of branched alkyl groups ($(CH_2)_mCH(CH_3)_2$) were used as R^1, selectivity showed a maximum ($R = 21.2$, $V = 87\%$) at $m = 2$. Such a remarkable dependence of selectivity on the substituent R^1, however, was not observed for system A where a series of straight-chain alkyl groups ($(CH_2)_nCH_3$) were used as R^1:

the product ratio (*R*) changed slightly from 0.3 to 0.5 except for the case $n = 2$. The data in Fig. 1 suggest the importance of intermolecular association in controlling chemical reactions through small changes in the structures of reacting molecules.

Figure 2*a* illustrates the plots of *R* at 35 °C against dielectric constants (ε), a measure of polarity of solvents. Figure 2*a* shows that with the associating system having the isopentyl group as R^1 in II (system A_a), selectivity decreased in going from 80% to 90% and 70% aqueous acetonitrile and from 80% acetonitrile to 80% dioxane and 80% *N,N*-dimethylformamide (DMF) with the maximum at 80% acetonitrile ($\varepsilon = 42.6$). The marked dependence of selectivity on ε led us to examine whether the thiol I or II associates in benzene or in acetonitrile—using a differential vapour pressure method. The degree of association (*f*), obtained by dividing the stoichiometric mole fraction of the solute by the effective mole fraction of the solute, was 1.31, 1.80, and 1.77 in benzene at 36 °C for I (0.01 M), II_a (0.04 M, $R^1 = isoC_5H_{11}$), and II_b (0.04 M, $R^1 = n\text{-}C_5H_{11}$), respectively. In contrast, in acetonitrile *f* was reduced to 1.04, 1.08, and 1.03 at 36 °C for I (0.02 M), II_a (0.04 M), and II_b (0.04 M), respectively. These data clearly indicate that *f* is reduced with increasing solvent polarity[7]. Accordingly, it is suggested that moderately weak association is essential for effective recognition between molecules, strong association being not essential. When the associating system contained the *n*-pentyl group as R^1 (system A_b), however, *R* had a minimum, though selectivity in this case was far less sensitive to ε than that with system A_a. In sharp contrast, with the non-associating system with the isopentyl group as R^2 in IV (system N_a) *R* showed a very slight variation from 2.5 to 1.8. This control experiment demonstrates that in the absence of intermolecular association, the three disulphides are formed in a nearly statistical ratio.

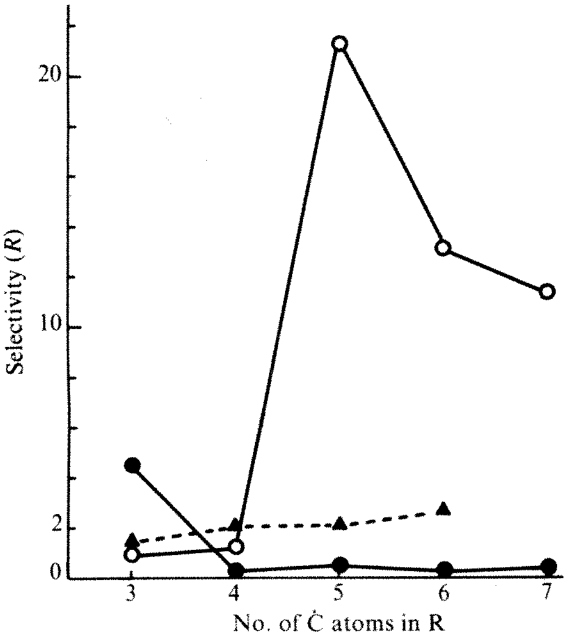


Fig. 1 Dependence of selectivity (*R*) in oxidation of a pair of the thiols (I and II or III and IV) on the structures of R^1 or of R^2. The two thiols (0.5 mmol each) were allowed to react with O_2 in 12.5 ml of 80% aqueous acetonitrile (v/v) at 35 °C in the presence of Et_3N (5×10^{-2} mmol) as a catalyst. Satisfactory spectroscopic data were used to support the assignment of new compounds. When the oxidation was completed, yields of V and VI were determined exactly by the use of their absorption at 310 nm after separation by thin-layer chromatography for system A, and determined gravimetrically after separation by preparative thin-layer chromatography for system N. ○, System A (R^1, branched alkyl groups); ●, system A (R^1, straight-chain alkyl groups); ▲, system N (R^2, branched alkyl groups).

In system A_a a plot of selectivity in 80% acetonitrile against temperature showed a peak of maximum selectivity at 35° C (Fig. 2*b*). In system A_b, however, the pattern of temperature dependence did not correspond to that of solvent polarity—*R* changed progressively over a wide range (0.13—2.3).

Of particular interest is the concentration dependence of selectivity with system A_a in 80% dioxane at 35 °C, represented by a sigmoid-like curve similar to the substrate concentration–reaction rate profiles observed with allosteric enzymes (Fig. 2*c*). In the case of systems N_a and even A_b, however, *R* remained almost constant with concentration.

In considering the significance of intermolecular association, it should also be noted that the product ratio (*R*) approaches about 2, the statistically expected ratio, in all systems in conditions unfavourable for hydrogen bonding, that is, in a polar solvent (for example *N*-methylformamide, NMF), at a higher temperature (70 °C), or at a lower concentration (1×10^{-3} M). It is clear from the results described here that environment dependence of selectivity is very sharp only in the case of system A_a among the systems examined.

All the results obtained from system A can be said to represent kinetic control on the basis of the following observations. First, the product composition does not change as the reaction proceeds, as is evident from the fact that the yields of V and VI during the period of the oxidation are 6.3% and trace, 15% and about 0.7% ($R = 21$), 23% and 1.1% ($R = 20.9$), 43% and 2.0% ($R = 21.5$), respectively, for system A_a and 2.2% and 4.4% ($R = 0.50$), 4.8% and 9.4% ($R = 0.51$), 14% and 27% ($R = 0.52$), respectively, for system A_b. System A_a required 2.5 h for complete oxidation to give 87% V and 4.1% VI ($R = 21.2$), whereas system A_b required 3.5 h to give 20% V and 39% VI ($R = 0.51$). Second, a thiol–disulphide exchange reaction occurred slowly in 80% acetonitrile at 35 °C in the presence of a catalytic amount of triethylamine under argon. The yields of V and VI at the same reaction time as with the above oxidation were 24% and 22%, respectively, for system A_a ($R = 1.1$, reaction time = 2.5 h) and 3.9% and 40%, respectively, for system A_b ($R = 0.098$, reaction time = 3.5 h), indicating that the product distribution in the exchange reaction differs entirely from that in the oxidation. The disulphide–disulphide exchange reaction did not take place to any appreciable extent in 80% acetonitrile at 35 °C in the presence of a catalytic amount of both I and triethylamine under argon[8].

System A was heterogeneous in some cases. However, the product ratio could not be ascribed to differential solubilities of the thiols and the product disulphides for the following reasons. First, the experiments on concentration dependence shown in Fig. 2*c* were carried out in completely homogeneous conditions, but the selectivity showed a sigmoid-like curve in the case of system A_a. Second, in 80% dioxane, 80% DMF, and NMF system A_a was also completely homogeneous at 35 °C during the oxidation, but *R* varied markedly from 14 to 1.9 (Fig. 2*a*). Third, in 80% acetonitrile a pair of the thiols I and II_a were completely soluble in the 25–70 °C range, the thiols I and II_b being also soluble at 35 and 70 °C (Fig. 2*b*). Further, the unsymmetrical and symmetrical disulphides derived from I and II were very stable in the reaction conditions, the unsymmetrical disulphides being more soluble in ordinary solvents than the symmetrical disulphides. These findings show conclusively that the selectivity is neither controlled by solubility differences between the thiols and disulphides and between disulphides V and VI nor by whether the reaction is heterogeneous or homogeneous[9].

Although the detailed nature of the specific interactions between I and II has not yet been made clear, the following observation suggests the presence of such interactions in system A_a. Plots of *R* against the mole ratio of I and II_a showed a maximum rise of *R* in a 1:1 mixture of I and II_a, indicating that I and II_a seem to form 1:1 weak complexes with each other, but not to complex with themselves. This type of complex is apparently stabilised by such interactions as those between the

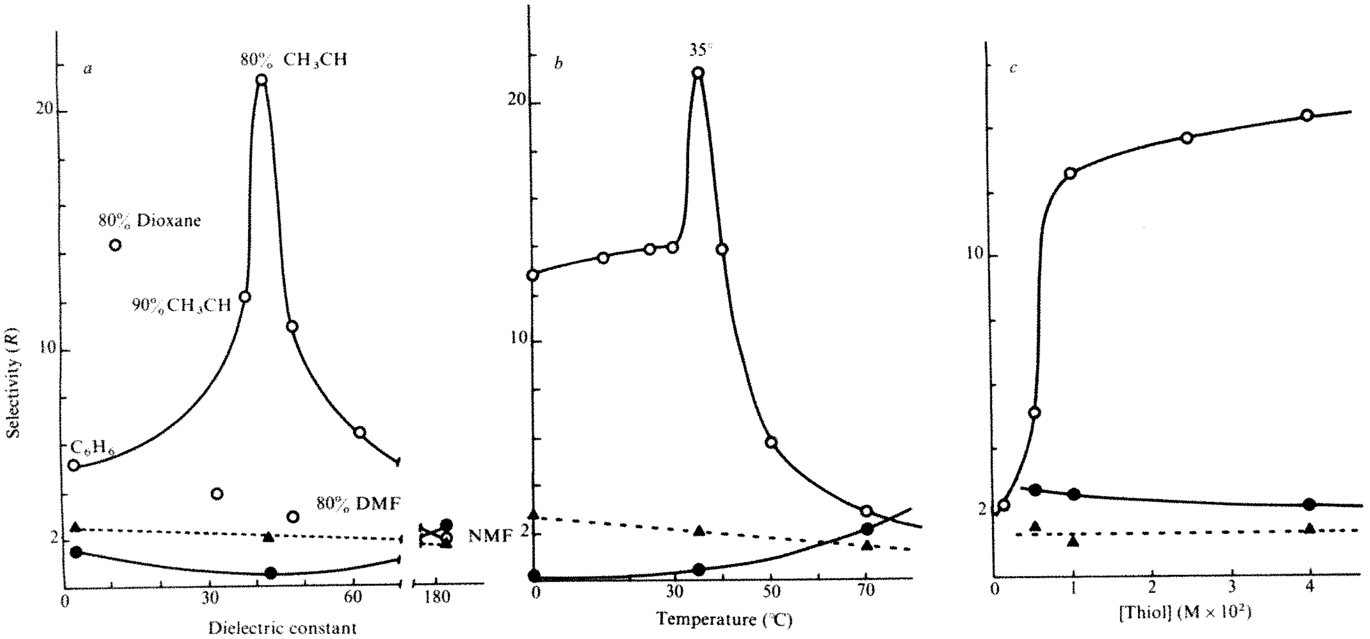


Fig. 2 Dependence of selectivity (R) in oxidation of a pair of the thiols (I and II or III and IV$_a$ (R^2 = isoC_2H_{11})). *a*, Dielectric constants of solvents (12.5 ml) at 35 °C; *b*, on reaction temperature in 12.5 ml of 80% acetonitrile, and *c*, on concentration of each thiol in 80% aqueous dioxane at 35 °C. The oxidations with O_2 were performed by using the same molar amounts of the thiols and Et_3N as described for Fig. 1. The series of solvents used to study solvent polarity dependence was (in order of increasing dielectric constant): benzene, 80% aqueous dioxane, methanol, 90% and 80% aqueous acetonitrile, 80% aqueous DMF, 70% and 50% aqueous acetonitrile, and NMF. ○, System A_a; ●, system A_b; △, system N_a.

aromatic ring in I and the branched alkyl group in II, in addition to intermolecular hydrogen bonding[10] between the two respective acylurea bonds in I and II. We conclude from our studies that both intermolecular association and geometrical shape are required for molecular recognition in chemical reactions.

Our results suggest that the use of appropriate model compounds which can associate and are of relatively low molecular weight should increase understanding of the problem of molecular recognition.

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Effects of cyclic AMP pulses on adenylate cyclase and the phosphodiesterase inhibitor of *D. discoideum*

STARVATION triggers the differentiation of *Dictyostelium discoideum* amoebae to aggregation competence. The differentiated cells orientate and migrate towards attracting centres, presumably consisting of amoebae which autonomously and rhythmically emit cyclic AMP as a chemotactic signal. Signals, emitted with a periodicity of 5–8 min (refs 1–3, 5), are presumably generated by rhythmic changes in adenylate cyclase activity[6], levels of which dramatically increase during the first few hours before the expression of aggregation competence[7,8]. Cellular responses to external cyclic AMP are probably mediated by plasma membrane receptors[9]. When stimulated by a pulse of cyclic AMP, amoebae amplify[10] and relay[1,5,10,11] the chemotactic signal, which results in a coordinated aggregation. The decay of the chemotactic signal is determined by the activity of the extracellular cyclic AMP phosphodiesterase[12,13] whose activity is modulated by an inhibitor excreted by cells during the early hours of starvation[14]. Cyclic AMP pulses have recently also been shown to regulate the differentiation of cells to aggregation competence[15]. To determine their mechanism of action, we examined the influence of applied cyclic AMP pulses on the two earliest biochemical events implicated in the control of cyclic AMP levels, the rise in adenylate cyclase activity and the appearance of the extracellular phosphodiesterase inhibitor. The results show that adenylate cyclase may exist in active or inactive forms. Applied cyclic AMP pulses seem to induce cell differentiation by activating the enzyme. They also are shown to repress the production of the phosphodiesterase inhibitor.

The changes in adenylate cyclase and the extracellular phosphodiesterase inhibitor which occurred when amoebae were starved in the presence or absence of cyclic AMP pulses are shown in Fig. 1. These changes are compared with the development of aggregation competence and the increases in cyclic AMP binding sites[9,16] and phosphodiesterase[16,17] which accompany it. In this experiment unpulsed amoebae were aggregation competent after 6–7 h of starvation, when they showed high levels of intracellular phosphodiesterase activity and cyclic AMP binding sites (Fig. 1*a*). When cells were pulsed with cyclic AMP, high levels of these two parameters were observed after 4.5–5 h of starvation, when they expressed aggregation competence. Assays of adenylate cyclase showed that the time course of enzyme induction was unaltered by cyclic AMP pulses: with pulsed and unpulsed cultures, activity first rose after 2 h of starvation and was maximal after approximately 5 h.

Determinations of the levels the extracellular phosphodiesterase inhibitor excreted by pulsed cells showed that they produced approximately 4% of the inhibitor produced by untreated cells (Fig. 1*b*). Incubation of amoebae in buffer containing 10^{-3} M cyclic AMP, delayed the appearance of the inhibitor by 1–2 h. Inhibitor was produced again when the added cyclic AMP had been hydrolysed by the extracellular phosphodiesterase (data not shown). The efficiency of 10^{-7} M cyclic AMP, when administered as pulses, in repressing inhibitor production may suggest that their effects are mediated by the increased cellular cyclic AMP levels obtained during signal amplification.

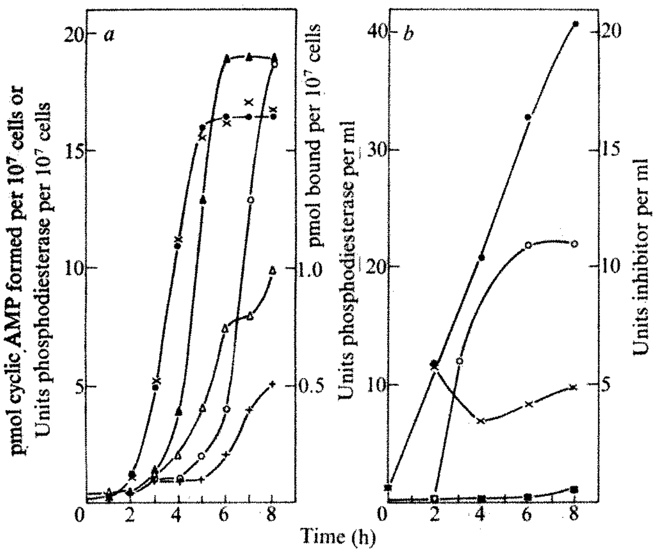

Fig. 1 Changes in adenylate cyclase, cyclic AMP binding sites, phosphodiesterase, and the phsophodiesterase inhibitor in cyclic AMP-pulsed and -unpulsed cells. Ax2 amoebae, harvested during exponential growth in HL-5 media[21], were washed twice with 17 mM phosphate buffer *p*H 6.2 and starved in that buffer as agitated suspensions[22] at a density of 10^7 cells ml^{-1}. Treatment of amoebae with 10^{-7} M cyclic AMP pulses was begun immediately after transfer of cells into buffer (t_0). The technique has been described in detail elsewhere[15]. Control cells were starved in the absence of applied pulses. At the indicated times, aliquots were taken and assayed for adenylate cyclase[7], cellular and extracellular phosphodiesterase activities[17] and the levels of cyclic AMP binding sites[23] and extracellular phosphodiesterase inhibitor[14]. Preparation of samples for inhibitor assays involves heating for 10 min at 80 °C (ref. 14). Although this treatment has been shown to result in a quantitative recovery of the inhibitor (independant of its interaction with the extracellular phosphodiesterase)[14], this was verified further for our experimental conditions where phosphodiesterase was present in large excess of inhibitor. Units of phosphodiesterase activity are defined as nmol cyclic AMP hydrolysed per min at 30 °C. The differentiation of cells to aggregation competence was followed by plating aliquots of cells on to Falcon tissue culture dishes at hourly intervals and observing cell morphology and formation of EDTA resistant cell contacts[22]. *a*, Adenylate cyclase of control cells (×) and of pulsed cells (●); cellular phosphodiesterase of control cells (○) and of pulsed cells (▲); cyclic AMP binding sites of control cells (+) and of pulsed cells (△). *b*, Extracellular phosphodiesterase of control cells (×) and of pulsed cells (●); phosphodiesterase inhibitor of control cells (○) and of pulsed cells(■).

Extracellular phosphodiesterase levels increased 4–5-fold during the time cells were stimulated by cyclic AMP pulses (Fig. 1*b*). This increase results from both a rise in enzyme production[17] and the decrease in the levels of the enzyme inhibitor. The dual control of cyclic AMP pulses on phosphodiesterase and its inhibitor assures the levels of extracellular phosphodiesterase required for cell differentiation (M.D. *et al.*, in preparation). Since amoebae feed on bacteria and therefore do not all begin to starve at the same time, the presence of the inhibitor during the early hours of starvation could assure that no aggregation occurs until the majority of the cell population produces the chemotactic signal. At that time, inhibitor production would be arrested and the phosphodiesterase induced.

The results described in Fig. 1 suggest that the rise in adenylate cyclase is not directly correlated with the expression of aggregation competence. This is further supported by the observation that unpulsed cell populations, which became aggregation competent after different time periods (5, 8 or 12 h), all increased their adenylate cyclase activities during the first few hours of starvation. In all these cases, applied cyclic AMP pulses did not alter the time course of the changes in adenylate cyclase but shortened the interval between the increase in this activity and the expression of aggregation competence and its related biochemical parameters. Cells may maximally increase their levels of adenylate cyclase during the first few hours of starvation but not display aggregation competence until many hours later because the enzymic activity assayed in cell lysates becomes functional in intact cells only after a delay. The following experiments support this hypothesis: Cells which became aggregation-competent within 5 h of starvation showed the periodic changes in light scattering correlated with changes in cyclic AMP synthesis and excretion[18] after 2–2.5 h. In contrast, cells which aggregated after 10–14 h did not show such behaviour during the first 6 h of starvation. Measurements of cyclic AMP produced by these cells showed that they did not synthesise cyclic AMP during the first 6 h of starvation. (Table 1, experiment 1). The levels of cyclic AMP synthesised by cells which were aggregation competent after 5–6 h of starvation are also presented (experiment 2).

Table 1 Cyclic AMP levels of late and early differentiating amoebae

	Cyclic AMP (pmol per 2 × 10^7 cells)	
Time of starvation (h)	Experiment 1	Experiment 2
2	0.17	3.5
4	0	4.0
5	.01	5.5
6	0	4.2

To initiate cell development, amoebae were washed twice with Volvic mineral water and resuspended in that water at a density of 10^7 cells ml^{-1}. No phosphate, which interferes with the assay for cyclic AMP, was added. The times at which cells became aggregation competent were the same as those which were starved in phosphate buffer (unpublished observations). Cells were starved as agitated suspensions[22] and at various times, 5 ml aliquots were added to 0.25 ml 100% TCA and immediately frozen in dry-ice ethanol. The procedure for sample preparation for cyclic AMP assays has been described[19]. Assays were performed using the protein kinase kit purchased from Amersham. In these experiments, cells collected from exponential growth (2–5 × 10^6 cells ml^{-1}) consistently became aggregation competent after 5 h of starvation. Therefore, to obtain cells which differentiated after a delay of 12–14 h, we harvested amoebae from early stationary phase cultures (experiment 1). It has been shown that such cells require longer periods of starvation in order to aggregate[24] but can be induced to differentiate within 8 h if pulsed with cyclic AMP[25]. It should be pointed out that the values for cyclic AMP levels produced by cells which become aggregation-competent after 5 h are minimum estimates because of the high levels of phosphodiesterase produced by differentiated cells.

Several arguments suggest that applied cyclic AMP pulses stimulate cell differentiation by activating adenylate cyclase rather than by merely replacing the endogenously produced chemotactic signal. Cells which aggregated after prolonged periods of starvation were, when pretreated with cyclic AMP pulses, induced to aggregate and therefore to produce autonomously the chemotactic signal after 6-7 h. An increase in cellular cyclic AMP levels resulting from signal amplification seems to mediate the induction of phosphodiesterase[17] and the repression of the enzyme inhibitor (described here) by cyclic AMP pulses. The stimulatory and inhibitory effects of various compounds on cell differentiation have been correlated with respective increases and decreases in cellular cyclic AMP[19] (P. Brachet and C.K., in preparation). Also Roos and Gerisch have recently shown that a transitory 2-fold stimulation of adenylate cyclase can be elicited by a pulse of cyclic AMP[20]. It is possible, however, that the activation of adenylate cyclase by external cyclic AMP pulses may not be the sole pathway by which pulses induce cell differentiation.

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Significance of glucose oxidase in lignin degradation

GLUCOSE oxidase (EC 1.1.3.4) is commonly found in the stagnant substratum of lignocellulose degrading fungi accompanying phenol oxidases, cellulases and glucosidases[1–4], where the steady-state supply of available oxygen is undoubtedly very limited[5]. Yet oxidants other than oxygen have heretofore received little attention as potential co-substrates of this enzyme despite its poor affinity for oxygen[4,6]. Spectrophotometric studies reported here on the interaction of glucose oxidase with transitory quinone and free-radical lignol oxidation products demonstrate that these intermediates are capable of serving as co-substrates of the reduced flavoenzyme at low oxygen tensions. Evidence is presented which suggests that glucose oxidase operates in concert with laccase, the principal phenol oxidase of *Polyporous versicolor*[3,7], a typical 'white-rot' fungus noted for its capacity to degrade lignin[1,8–13], to maintain a laccase–glucose: quinone oxidoreductase cycle in the fungal substratum by which lignol substrates may become highly oxygenated and ultimately degraded.

Table 1 Laccase and glucose oxidase activities in culture extracts of *P. versicolor**

Substrate	mU ml^{-1} Laccase	Glucose oxidase
Chlorogenic acid	22–49	–
Glucose	–	68–164

*Extracellular enzyme activity in crude 10,000*g* supernatant. Cultures were grown on sterile semi-solid rye grass media[16] and cells collected 10–20 d after innoculation. Laccase and glucose oxidase were assayed polarographically[17] at 37 °C on a Gilson Oxygraph equipped with a water-jacketed Clark electrode cell. Routine assays were with 0.1–0.5 ml enzyme in 50 mM potassium acetate buffer, *p*H 5.0, and 10 µmol of substrate in a total volume of 1.8 ml. Enzyme units are expressed as µmol min^{-1} of substrate turned over at 37 °C and represent the range seen in assaying five separate colonies of *P. versicolor*.

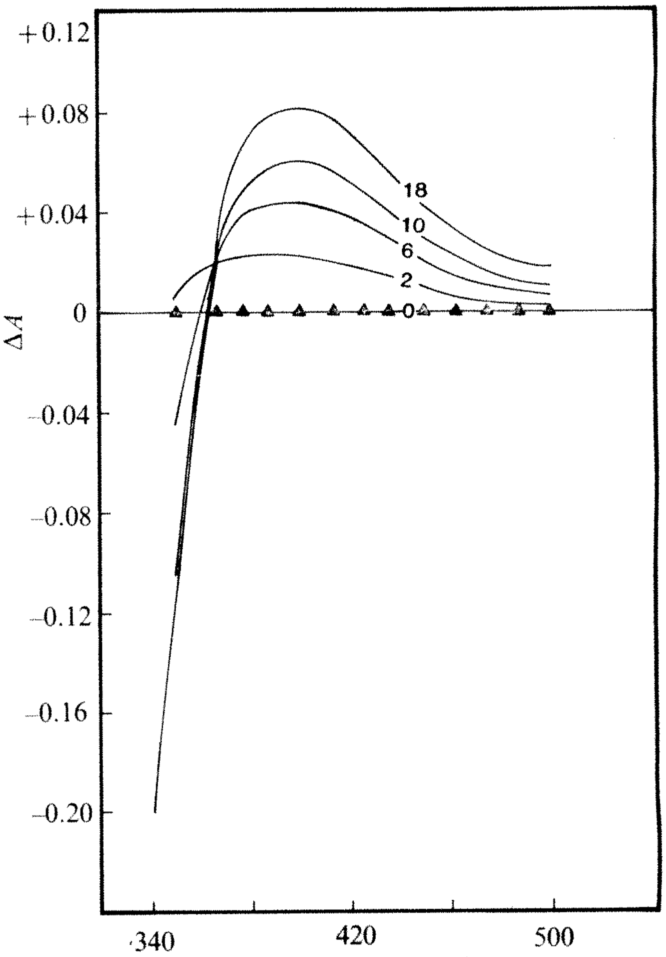

Fig. 1 Generation of quinoid intermediates *in situ* from chlorogenic acid by *P. versicolor* laccase. Sample and reference quartz cells contained 3.0 ml of 0.33 mM chlorogenic acid in 50 mM potassium acetate buffer, *p*H 5.0. At *t* = 0, 20 µl of stock enzyme (compare with Table 2) was added to the sample cell and the visible spectrum scanned on a Model 11 Carey spectrophotometer. Continuous scans were then made at 2, 6, 10 and 18 min after enzyme addition as indicated. Time-dependent difference spectra were plotted as the difference in absorption between the sample at time, *t*, following initiation of the reaction, and *t* = 0. —, Difference spectrum seen after 20 min when the same reaction was run with 0.15 mM sodium azide included in the assay mixture.

Five 'white-rot' fungi, *P. versicolor, P. abietnus, Poria sabicita, Phoriata adiposa* and *Formes fermentarius,* were chosen in initial screening trials for their capacity to produce extracellular phenol oxidases while growing on a semi-solid rye grass substratum supplemented with minimal salt media. Crude enzyme was recovered following filtration and centrifugation of initial slurries of the mycelial straw substrata prepared in 50 mM potassium acetate buffer, *p*H 5.0. Of the five fungi, *P. versicolor* grew most profusely on the semi-solid medium, and crude enzyme extracts of its substratum were especially rich in laccase and glucose oxidase activity (Table 1).

Dichlorophenol-indophenol (DCIP) was readily oxidised by enzyme mixtures exposed to air, as shown by a sharp blue shift in its adsorption maximum from 575 to 510 nm and a concomitant consumption of dissolved oxygen; it was not metabolised in solutions purged of oxygen and kept under nitrogen (Table 2). Addition of glucose to DCIP-enzyme mixtures under a nitrogen atmosphere brought about complete reduction of DCIP, however, and the readmission of air to these solutions resulted in the reappearance of the DCIP absorption spectrum. Interconversions between DCIP and its dihydro-form were induced through several cycles by appropriate adjustments of the oxygen tension, but continuous exposure of DCIP–enzyme mixtures to atmospheric oxygen resulted in irreversible losses of substrate no longer susceptible to further reductive cycles.

Model quinoid intermediates were also produced *in situ* by mixing laccase from test fungal extracts with the dilignol substrate, chlorogenic acid, as seen by a time-dependent, increased absorption maximum between 380 and 400 nm in the reaction mixture (Fig. 1). Excluding oxygen from the enzyme–substrate mixture, or blocking laccase activity by the addition of azide, prevented formation of this spectrum. These results link the appearance of the 380 nm absorption peak with *in situ* generation of quinoid intermediates.

Figure 2a demonstrates a dual requirement for both glucose oxidase and glucose in bringing about reduction of *in situ*-generated quinoid intermediates. Although most of the quinoid spectrum disappeared with glucose–glucose oxidase additions, there was a gradual accumulation of material less susceptible to reduction. Azide addition following initial generation of quinoid intermediates blocked further quinoid formation, and a slight decrease in the 380-nm absorption peak occurred thereafter (Fig. 2*b*). Subsequent additions of glucose and glucose oxidase to the azide treated samples caused only a slight further decrease in absorption. These latter results indicate a rapid turnover of quinoid intermediates. The residual, non-reduceable background absorption at 380 nm seems to coincide with a shift in the average absorption spectrum towards longer wave lengths (compare with Fig. 1) and probably reflects concomitant and irreversible structural rearrangements among transition state intermediates (for example, quinoid condensates, polymerisations) (ref. 13).

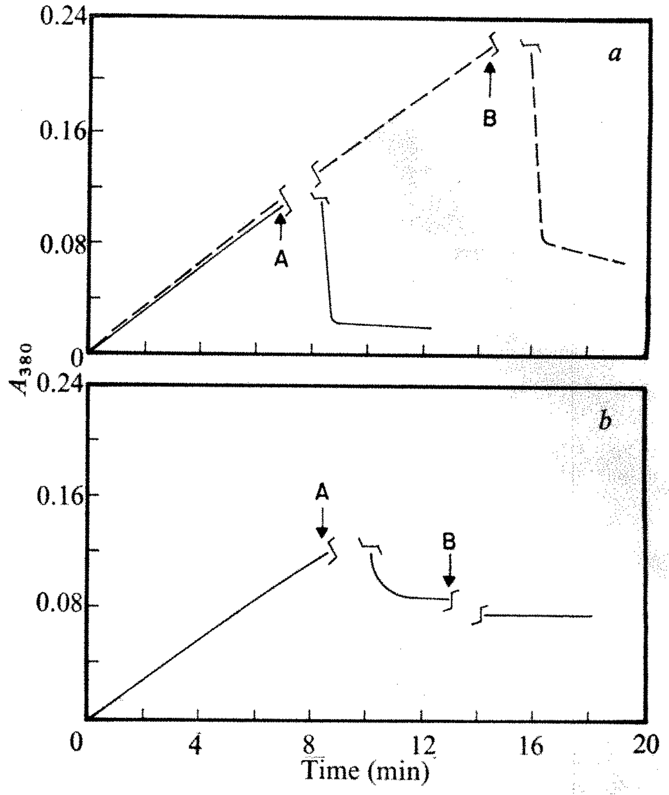

Fig. 2 Effect of glucose–glucose oxidase additions on the reduction of *in situ* generated transistory quinoid intermediates of chlorogenic acid. Quinoid intermediates were generated as described in Fig. 1 and their appearance monitored with time at 380 nm. *a*, Two separate experiments are shown : 3.3 mM glucose was included in the initial reaction mixture of the first experiment (—) and omitted from the second (- - -). About 7 min after addition of laccase each experiment was briefly interrupted (point A) with addition of 100 μl of commercial (Sigma) glucose oxidase (120 units). Immediately thereafter the 380 nm absorption was again monitored with time. The experiment lacking glucose failed to register a decrease in 380 nm absorption until glucose (1.0 μmol) was also introduced to the mixture approximately 8 min thereafter (point B). *b*, The same experiment as in (*a*) was repeated with 3.3 mM glucose in the initial reaction mixture, except 10 μl (45 μmol) of sodium azide was added to the sample cell 7 min after initiation of quinoid production by laccase (point A); approximately 6 min thereafter glucose oxidase (120 units) was added to the sample and the 380 nm absorption again monitored as shown.

Table 2 Effect of oxygen and glucose on the laccase–glucose: quinone oxidoreductase cycle

	Substrate	A_{575}	Comments
Experiment 1	DCIP	1.00	Incubated 30 min under nitrogen
		0.56	5 min after admission of air oxidised DCIP products seen in absorption spectrum
Experiment 2	DCIP+glucose	0.00	Incubated 30 min under nitrogen
		1.00	2 min after admission of air absorption spectrum identical with DCIP spectrum.

0.5 ml fungal enzyme extract (approximately 0.3 units laccase; 0.5 units glucose oxidase) and 0.33 μmol DCIP were mixed together in a final volume of 3.0 ml 50 mM potassium acetate, *p*H 5.0. Nitrogen was bubbled through the enzyme–substrate mixtures except as noted. The absorbance of DCIP at 575 nm was determined on a Model 11 Carey spectrophotometer; visible spectra of the reaction mixtures were also taken at selected intervals between 375 and 700 nm. Fungal enzyme extracts were prepared by extracting the semi-solid substratum of *P. versicolor* cultures in 50 mM potassium acetate buffer, *p*H 5.0, filtering the crude slurry through cheese cloth, and centrifugation to yield a cell-free 10,000*g* supernatant. Stock enzyme was recovered as an ammonium sulphate pellet (0–90% saturation).

The enzymatic oxidation of glucose by glucose oxidase is known to occur in two steps: (1) oxidation of glucose to gluconolactone through the transfer of two electrons to a flavin prosthetic group on the enzyme; and (2) reoxidation of the reduced flavoenzyme through a second two electron transfer to a suitable electron acceptor[4,14], and thus the capacity of oxidants to participate in the enzymatic oxidation of glucose rests in their ability to accept electrons from the reduced enzyme. It is noteworthy that the K_m of glucose for glucose oxidase decreases from 4.2 to 1.9 μM upon decreasing the oxygen tension in the gas phase from

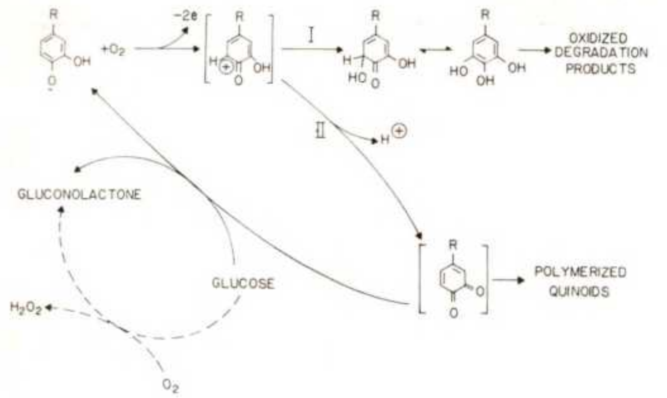

Fig. 3 Schematic flow diagram of postulated laccase–glucose: quinone oxidoreductase cycle and its significance in the degradation of lignol substrates.

20 to 5%, respectively, but that glucose oxidation is less than half V_{max} at atmospheric oxygen tensions in the presence of saturating concentrations of glucose[4,6,15]. Oxygen is thus a poor substrate of glucose oxidase, whereas glucose is an excellent substrate (for example, reductant) whether oxygen is present or absent from solution. Moreover, at physiologically low oxygen tensions the ability of quinone or free-radical lignol intermediates to compete with oxygen as oxidants of glucose oxidase would be expected to be of greater significance because of an improved ratio of quinoid concentration over that of oxygen. The experimental results of this study corroborate these observations.

Figure 3 outlines a metabolic role for glucose oxidase which may provide a rationale for laccase–glucose: quinone oxidoreductase cycling. Initial abstraction of electrons from a lignol substrate by laccase transforms the substrate into an electron deficient transition state intermediate, which may undergo nucleophilic attack by hydroxylation as illustrated by route I of Fig. 3. On the other hand, an electron shift and loss of proton may occur to yield a transitory quinone intermediate as shown by route II of Fig. 3. Without glucose: quinone oxidoreductase activity, transition state intermediates produced by laccase would accumulate, condensing and polymerising with products secreted into the microbial substratum. Glucose oxidase may thus serve two functions in the substratum: (1) to protect the metabolic processes of the microbial cell from toxic accumulations of quinoid intermediates; and (2) to greatly improve the efficiency of lignol oxidations by channeling substrates through productive degradative routes.

In view of these observations, the physiological significance of oxygen as a co-substrate of glucose oxidase is worth re-examining, possibly as a potential modifier of glucose: quinone oxidoreductase activity, because of its capacity to compete as an oxidant of glucose oxidase, and in the process to uncouple normal channeling of lignol oxidation products through the laccase–glucose: quinone oxidoreductase cycle.

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Unusual mitosis in the red alga *Porphyridium purpureum*

Porphyridium purpureum (Bory) Drew *et* Ross (Syn.: *P. cruentum* Nägeli) is a classical laboratory organism and one of the few red algae easily grown in large quantities. This would make it an obvious candidate for investigation of red algal mitosis with the electron microscope. The nuclei are, however, quite small and fast dividing, so that previous attempts remained apparently unsuccessful[1,2] and left the much more complex species *Membranoptera platyphylla* the only red alga where mitosis had been satisfactorily described[3]. We are now able to report that mitosis in *Porphyridium* is not only quite distinct from that in *Membranoptera* but also presents an unique association of microbodies with the spindle poles.

In order to increase the chance of observing mitotic figures, a culture of *P. purpureum* strain 161 of the University of Indiana culture collection grown in artificial seawater[4] was synchronised by a 12 h light–12 h darkness cycle according to Gense *et al*[5]. modified by Knappe[6]. Cells collected the fourth day after the beginning of synchronisation, 8 h after the beginning of the light period (the best time for mitosis as estimated from cell count curves and observation of feulgen-stained material), were fixed with glutaraldehyde, postfixed with osmium, sectioned and stained with uranyl acetate and lead.

Despite the fact that several hundred cells were examined,

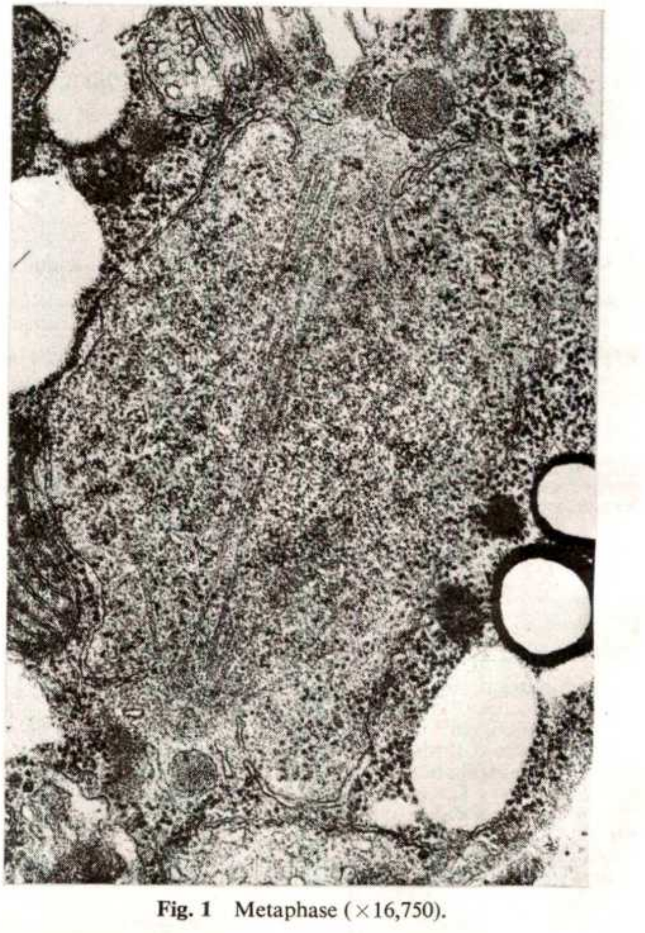

Fig. 1 Metaphase (×16,750).

only four were in metaphase and for only one did the plane of the section include both spindle poles (Fig. 1). The nuclear envelope is well preserved except for large polar fenestrae where microtubules end in a ribosome-free area delimited by fragments of endoplasmic reticulum and microbodies (one to three). No well defined pole body is apparent and the microtubules seem to be divided into a central bundle of interpolar ones and peripheral ones that end in small darker masses that might be badly condensed chromatin without kinetochores. The nucleolus is apparently dispersed, with some ribosome-like particles visible in the periphery of the nucleus.

A few tangential sections of nuclei that might be in prophase suggest that at that time an amorphous microtubule-organising centre might be present, to which extranuclear microtubules are connected (Fig. 2). This is however insufficient to demonstrate an extranuclear origin of the spindle.

Nuclear division and rebuilding of the nucleolus are performed before the onset of cytokinesis.

Microfilaments about 10 nm in diameter similar to those reported in other algal and fungal nuclei[7–9] have also been observed, but not in dividing nuclei (Fig. 3).

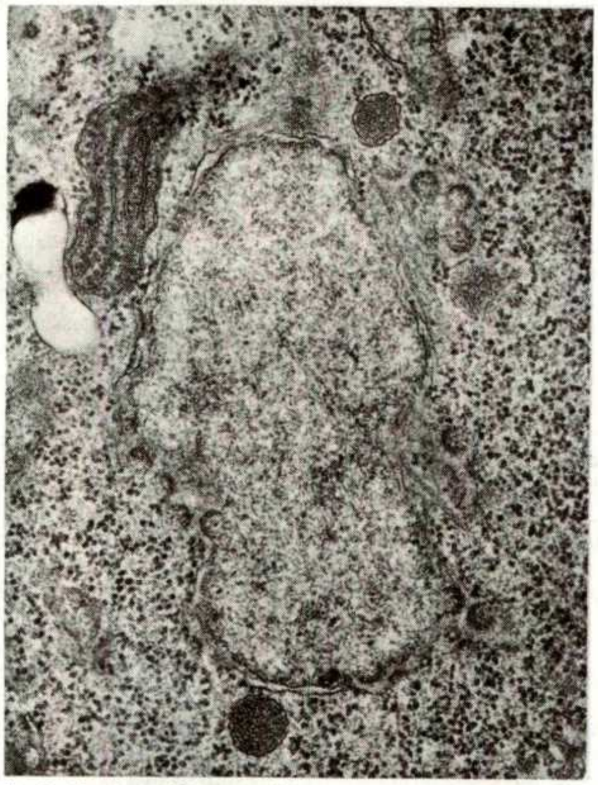

Fig. 2 Prophase (?); tangential section of the nucleus (×16,750).

It is notable that we have observed none of the figures previously interpreted as cytoplasmic invaginations in the nucleus during mitosis[1], although we have worked with the same strain. From further studies by those authors[2] it seems that those figures were attributable to a viral infection that did not manifest itself in our culture.

The reported nuclear behaviour of *Porphyridium* is quite distinct from that of the more complex red alga previously studied[3]. It lacks the polar rings and kinetochores (unless those structures have escaped our sections), the well condensed chromosomes and the doubling of the nuclear envelope by endoplasmic reticulum. The polar microbodies (a feature verified in three other nuclei) are as far as we know an unique feature. A microbody is pictured by Franke and Reau[10] near a dividing nucleus of *Phycomyces*, but other pictures seem to imply that this is not a regular feature. In *Pyramimonas*[11], a single large microbody has a peculiar behaviour during mitosis but might be more associated with the flagellar bases than to the nucleus.

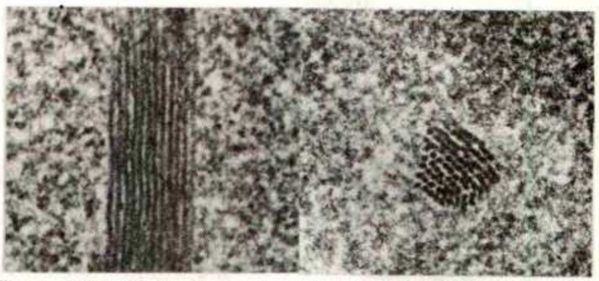

Fig. 3 Microfilaments in the nucleus: left, longitudinal section; right, transverse section (×33,500).

The microbodies are apparently similar to those previously studied in *Porphyridium* by Oakley and Dodge[12].

The large polar fenestra is rather similar to that of the Basidiomycetes *Poria latemarginata*[13] and the lack of condensed chromosomes is a typical feature of higher fungi but at the moment *Porphyridium* cannot be clearly related to any other organism on the basis of its mitosis[14–16].

Its mitosis is anyway not closer to that of Cryptophyceae[17] than that of *Membranoptera* and so does not change the situation in the controversy on possible relationships between Rhodophyceae and Cryptophyceae[18–19].

Studies of further red algae, especially the primitive ones would obviously be extremely interesting as the mitotic type of the group cannot be only characterised by what is known in *Membranoptera*.

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Carotenoid transformations during chloroplast development in *Scenedesmus obliquus* PG1 demonstrated by deuterium labelling

THE unicellular green alga *Scenedesmus obliquus* (Chlorophyta), when grown photosynthetically, contains pigments (chlorophylls and carotenoids) similar to those of the chloroplasts of higher plants[1]. Several mutant strains[1] have abnormal pigmentation[1]. One of them, PG1, when grown in the dark accumulates the acyclic carotenoids phytoene (7,8,11,12,7′,8′,11′,12′-octahydro-ψ,ψ-carotene), phytofluene (7,8,11,12,7′,8′-hexahydro-ψ,ψ-carotene) and ζ-carotene (7,8,7′,8′-tetrahydro-ψ,ψ-carotene) in place of cyclic carotenes and xanthophylls, and cannot make more than trace amounts of chlorophylls. The pigmentation of light-grown PG1, however, is virtually identical to that of the wild type. When dark-grown PG1 is illuminated, the content of acyclic carotenoids decreases and that of cyclic carotenoids increases[2]. Time-course studies[3] agree with the hypothesis that the cyclic carotenoids produced on illumination are formed from the acyclic precursors that accumulate in dark-grown cultures. It has not been possible to confirm this by radioisotopic labelling, but we have now done so using deuterium labelling. Our procedure also provides a general method for following the kinetics of formation of important chloroplast constituents during chloroplast development in this and other organisms.

Wild-type *S. obliquus* can be cultured (autotrophically) in deuterated medium (99+% D_2O; ref. 4). By culturing *S. obliquus* PG1 in the dark on ordinary (H_2O) medium and then transferring to D_2O and illuminating we have developed a method in which deuterium labelling is used to examine changes that occur in the carotenoid composition of the organism during illumination. *S. obliquus* strain PG1 was grown heterotrophically in the dark, in which conditions it accumulated ζ-carotene, phytoene and phytofluene as its main carotenoids[1]. The cells were harvested, resuspended in D_2O without added nutrients, and starved overnight by shaking in the dark to deplete starch reserves. A mixture of air–CO_2 (95 : 5) was then bubbled through while the cell suspension was illuminated for 4 h. The main cyclic carotenoids, α-carotene (β,ε-carotene), β-carotene (β,β-carotene), lutein (β,ε-carotene-3,3′-diol) and zeaxanthin (β,β-carotene-3,3′-diol) were subsequently extracted and purified[1] and examined by mass spectrometry. The important features of the mass spectrum of β-carotene are illustrated in Fig. 1 and discussed below; similar results were obtained for the other carotenoids. Three features in the mass spectra are particularly important.

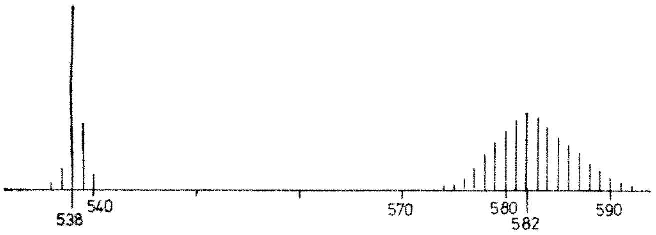

Fig. 1 Features of the high mass region of the mass spectrum of deuterio-β-carotene produced by *S. obliquus* PG1 grown in the dark and then resuspended in D_2O and illuminated.

(1) There was virtually no trace of the normal molecular ion of α-carotene or β-carotene (both $C_{40}H_{56}$) at *m/e* 536 or of lutein or zeaxanthin (both $C_{40}H_{56}O_2$) at *m/e* 568. There had therefore been virtually no synthesis of undeuterated cyclic carotenoids.

(2) In all cases by far the most intense ion in the high mass region corresponded to *m/e* (M+2) in the spectrum of the unlabelled carotenoid, that is at *m/e* 538 for the carotenes and 570 for the xanthophylls. This indicated that the most abundant molecular species in each case was one containing two deuterium atoms. The mass spectrum of recovered, uncyclised ζ-carotene showed only the normal molecular ion at *m/e* 540 ($C_{40}H_{60}$) with no ion at *m/e* 542 corresponding to a dideuterio species.

(3) The group of ions at approximately *m/e* 574–592 in the spectra of α- and β-carotenes and at approximately *m/e* 606–622 in the spectra of lutein and zeaxanthin, in each case accompanied by characteristic (M−deuterotoluene) fragmentations, indicated the presence of molecular species containing extensive deuterium labelling (average composition $C_{40}H_{10}D_{46}$ for carotenes and $C_{40}H_{12}D_{44}O_2$ for the xanthophylls). The relative intensities of these ion groups gave a measure (assuming similar volatilities for all the molecular species in the mass spectrometer ion source) of the extent of new synthesis of cyclic carotenoids from small molecules, for example CO_2 and D_2O occurring during illumination.

The cyclisation reaction of carotenoid biosynthesis (Fig. 2) is generally considered to be a proton-initiated process[5]. Thus if an acyclic precursor carotenoid is formed in ordinary (H_2O) medium and then cyclised in D_2O, one deuterium atom will be introduced at C–2 during each cyclisation, that is, bicyclic carotenoids will contain two deuterium atoms. The mass spectrometric assay therefore directly demonstrates that in greening *S. obliquus* PG1, α- and β-carotenes, lutein and zeaxanthin are all formed by cyclisation of existing acyclic precursors (for example ζ-carotene, presumably first desaturated to lycopene). Conclusions drawn previously[3] from the results of kinetic studies without isotopic labelling are therefore valid.

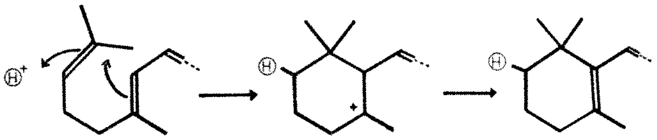

Fig. 2 Mechanism of the cyclisation reaction of carotenoid biosynthesis.

Perhaps the most important feature of this procedure is that, by mass spectrometric assay, molecules synthesised from preformed intermediates can be distinguished from molecules formed by new synthesis. Studies of the time-course of events occurring during chloroplast development are therefore possible. For example, when dark-grown PG1 is transferred to D_2O and illuminated, and aliquots of the greening cells are removed after various time intervals and the cyclic carotenoids are extracted and assayed by mass spectrometry, it is apparent that in the early stages of greening (up to 2 h) the cyclic carotenoids are derived mainly from the preformed cyclic intermediates, whereas after longer times the cyclic carotenoids are increasingly being produced by new synthesis. Note, however, that the greening process occurs much more slowly in D_2O than in H_2O.

This procedure clearly has potential as a general method for studying metabolic pathways or developmental processes that can be interrupted, for example by inhibition, mutation, lack of light energy, but can then be completed under appropriate conditions. If the first phase of the process (up to the interruption) takes place in ordinary (H_2O) medium and the second phase (after removal of the interruption) is allowed to proceed in D_2O or D_2O-based medium, then mass spectrometric assay of the type described above can be used to differentiate between compounds produced entirely during the first phase, entirely during the second phase, or, in appropriate cases such as cyclic carotenoids, formed during the second phase from intermediates produced during the first phase. The method can thus be used to obtain information about the origins of various chloroplast components, the time-course of their formation and their relative importance in the chloroplast structure, during development of algal chloroplasts. The method is also applicable to studies of the development of the photosynthetic apparatus in photosynthetic bacteria. We have used a related procedure to demonstrate the formation of cyclic carotenoids from accumulated lycopene in a *Flavobacterium* species and to determine the stereochemistry of the proton-initiated cyclisation reaction. (G. B., W. J. S. L., N. J. Patel and T. W. G., unpublished).

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reviews

Curious little book on rabies

George M. Baer

Rabies: The Facts. Edited by Colin Kaplan. Pp. 116. (Oxford University: Oxford and London; Corgi: London, 1977.) Hardback (OUP) £1.95 paperback (Corgi) 75 pence.

THIS is a curious little book on rabies. If it is intended for a scientific audience, it is the first such book I know of without references. If it is a book for the general public, it contains highly specialised information on nucleoproteins, structural proteins of the virus, and comments rather advanced for the average reader such as: "In their model of rabies virus, Vernon and his colleagues have depicted the larger of the two M proteins (M_1) as being adjacent to the ribonucleoprotein".

The book is divided into seven chapters, begining with a discussion of the world problem, and emphasising the under-reporting of the disease. Under-reporting has also been discussed in a notable 1967 report by Sanmartín *et al.* (Sanmartín, Correa, Dueñas & Muñoz, Memorias del Primer Seminario Nacional Sobre Rabia, Medellín, Colombia, 155–161, 1967) in which 1.7% of 1,596 autopsies in the University Hospital in Cali, Colombia, showed that death was due to rabies.

It is unfortunate that no maps or tables are included in a discussion of world rabies epidemiology, and that the description of the flourescent antibody technique is not accompanied by a diagram making it a little more intelligible for the layman. The technique has been of tremendous value in rabies diagnosis and investigations.

The descriptions of the disease attests to the terror that rabid dogs once instilled in the hearts of English countrymen, much as it does today in thousands of people in most developing countries. It has often been noted that rabies infection in the field frequently results in antibody production in a sizeable percentage of animals, much as described in this book. This includes animals such as insectivorous bats (Burns & Farinacci *J. Infect. Dis.* **97**, 211; 1955), mongooses (Everard, Baer & James *J. Wildlife Dis.* **10**, 190; 1974), dogs (Doege & Northrop *Lancet*, **ii**, 826; 1974) and other fairly resistant species. Much is made of the two human recoveries from rabies (Hattwick *et al. Ann. Intern. Med.* **76**, 931; 1972; Porras *et al. Ann. Intern. Med.* **85**, 44; 1976), as well as one purported carrier dog (Veeraraghavan *et al., Ann. Rep. Pasteur Institute of Southern India*, 66, 1968), yet it has not been pointed out that these are bizarre exceptions to the rule.

The chapter on rabies virus itself by Crick and Brown is a complete description of the morphology, chemistry and structural subunits of the virus, and of rabies-related viruses. It also covers replication of the virus, synthesis of virus components and other products from infected cells.

The chapter on human rabies includes various aspects of the disease in man, including susceptibility, symptomatology, diagnosis and prevention. An amazing anecdotal series of cases of rabies transmitted from man to man is succeeded by a statement that "... there is no completely documented case of man transmitting rabies to man", in which case it seems pointless to even mention the dramatic anecdotal cases. Another amazing statement is: "For reasons which are unknown rabies is up to seven times commoner in men than in women: this also applies to animals". This statement may be applicable to man (see review by Hattwick in *The Natural History of Rabies*, Academic, New York, 1975), but it is certainly not for animals. Verts (*The Biology of the Striped Skunk*, University of Illinois Press, Urbana, Chicago and London, 1967) writes that "Of the 20 rabid striped skunks caught in northwestern Illinois, 19 (95.0 per cent) were females". This type of information should not be included unless it is properly documented.

The chapter on rabies in animals includes a table listing incubation periods for various species, with errors: for example, incubation periods of more than 5 months have been reported for foxes (Sikes, *Am. J. vet. Res.* **23**, 1041; 1962) and periods of more than one year have been reported in cattle (Abelseth & Lawson, cited by Bell, *The Natural History of Rabies*, Academic, New York, 1975). Photographs of humans and animals with rabies, as well as electron micrographs of rabies virus are included in this chapter. There is rightful emphasis on the variability of the signs in dogs, and on the fact that relatively few develop furious rabies. It is again unfortunate that no references are included; for example, I am not acquainted with the outbreak of rabies reported in pigs. Other curious statements such as "Deer are as prone to rabies as cattle . . ." should be documented. The chapter ends with the astounding suggestion that "There is a potential danger here (that is, Britain) that rabies might enter this country and become enzootic in mink much the same way it has become established in the mongoose. . . " One wonders why this has not already occured in the numerous countries where rabies is enzootic and mink are plentiful.

The next two chapters are on fox behavioural ecology and the prospects of wildlife rabies in Britain. They include a description of fox behaviour and rabies in the fox, with summaries of studies done in Britain and other countries. These chapters are basically well presented and include much useful information. The possible transmission of rabies from cats to foxes is emphasised, without any explanation as to why this mode of transmission is believed to occur. In fact, Wachendörfer has shown (*Dt. tierärztl. Wschr.* **69**, 555; 1962) that the case of rabies transmitted from foxes to cats are closely related and the two epizootiological curves can be virtually superimposed.

The short chapter on rabies vaccines by Turner encompasses most of what is known today about human and veterinary vaccines, with emphasis on the difference between pre-exposure vaccination (such as vaccination of animals and of people at risk to exposure in the laboratory), and post-exposure vaccination. □

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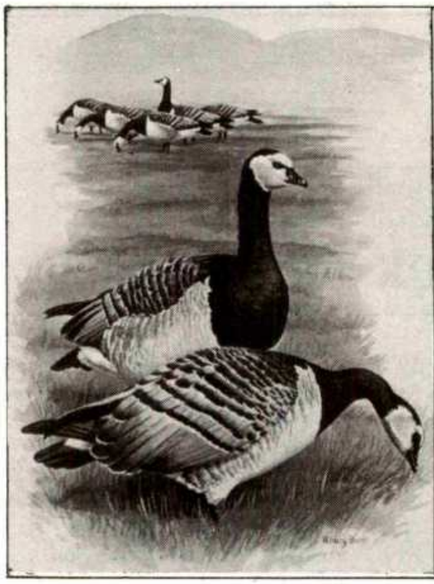
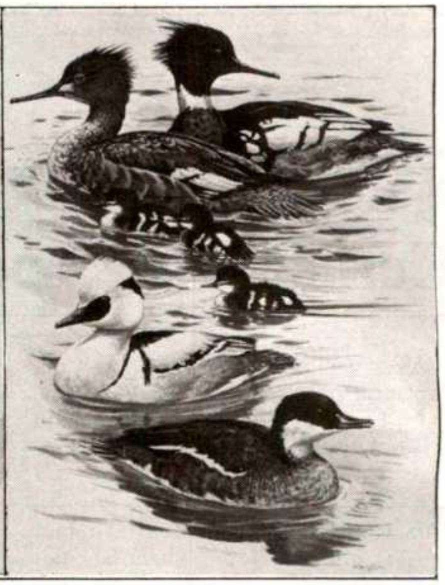
European wildfowl

Wildfowl of Europe. By Myrfyn Owen. Colour plates by Hilary Burn and foreword by Sir Peter Scott: Pp. 256. (Macmillan: London and Basingstoke, 1977.) £15.



Barnacle Goose



Red-Breasted Merganser (above) and Smew (below)

QUITE often popular books of this sort are written to support the illustrations and this may have been the original idea for this volume. The author admits that this book does not pretend to summarise all known information about wildfowl. He attempts to cover most aspects of the birds' lives unclouded by tedious detail and he confesses to a personal bias in what he has omitted. The first quarter of the book deals with taxonomy, waterfowl movement, populations dynamics, migration and man's relation to waterfowl. Helped as he has been by the scientific staff of the Wildfowl Trust, the author has included some new quantative information in these chapters. The final chapter of this section is spoiled by his unnecessarily chauvinistic attitude, which gives the reader the misleading impression that one can only see wildfowl in three localities in Britain—three reserves administered by his own organisation.

The remaining three-quarters of the book provides a brief history of all the species of wildfowl recorded in Europe. Many chapters contain new information resulting from research undertaken by the Wildfowl Trust: particularly valuable are the figures for European populations based on counts organised by the International Waterfowl Research Bureau, and the accounts of the feeding ecology of nearly all the main species of duck. But there is little about breeding behaviour in the individual accounts. Four useful appendices summarise information about nests, eggs and young of wildfowl, winter weights and measurements, winter food and waterfowl in captivity.

This volume is illustrated by coloured plates—for which incidentally there is no index or list—by Hilary Burn, a promising young bird painter whose work has developed rapidly. But because of her predeliction for the use of black to create shadows, many of the plates of the colourful species such as wigeon, pochard and mallard are sadly lacking in natural sparkle. The design of some of the plates could show more imagination and the scale is sometimes awry. Joe Blossom's line drawings are generally competent at underlining points made in the text.

Interesting though it is, I find it difficult to accept that this rather popular volume is really worth the £15 charged for it: while the wildfowl enthusiasts will buy it, the specialist would have expected something much more comprehensive. Costs might have been less if the margin had not been so wide—75 millimetres for a single page or 150 millimetres when the book is open.

Peter Conder

Peter Conder recently retired as Director of the Royal Society for the Protection of Birds.

Push-button biology

Human Biology: An Exhibition of Ourselves in the new Hall of Human Biology at the British Museum (Natural History), London. A permanent exhibition, admission free.

Human Biology: An Exhibition of Ourselves. Pp. 120. (British Museum (Natural History) and Cambridge University: London, 1977.) Hardback £5.00; paperback £1.95.

THE dinosaurs and whales, and halls of decorously deceased representatives of the animal kingdom, at the British Museum (Natural History) (BM (NH)) have been joined by an exhibition which requires more of the visitor than tireless legs and unglazable eyes. The Hall of Human Biology is the first fruit of the major reorganisation which is intended, during the next 30 years or so, completely to rearrange the museum's specimens. They have been presented for more than 100 years according to Victorian notions of the plan of the Creator, which are no longer deemed appropriate. In future visitors will be expected to discover as well as to learn, and the specimens will be accompanied by buttons to push, handles to turn and games to play. Although in Britain this represents a revolutionary new concept in museums, it has been flourishing abroad for some time in such centres as the Oregon Museum of Science and Industry in Portland.

In An Exhibition of Ourselves, which fills the Hall of Human Biology, visual, tactile and olfactory aids can be manipulated to reveal details of growth and development, and of the control of bodily functions and their responses to the external environment. Much of the material has been incorporated into the accompanying book, which consequently tells its story as much through pictures as through the text. In the tradition of such BM (NH) publications, the book can be used independently, although it follows closely the plan of the exhibition.

Willing learners, young or old, with some experience of biology textbooks should find that the exhibition usefully and enjoyably complements their knowledge. For them animated models will explain homeostatis and neuromuscular control; a slide show will clarify the events of gestation and birth, and an optical illusion will tell something about perception. Afterwards *Human Biology* will remind them of the concepts they have encountered.

Visitors with little or no aquaintance with biology, however, may have to concentrate quite hard on the exhibition. And that could be difficult when the maze of exhibits is swarming with exuberant youth apparently determined to flash all lights and ring all bells at once. But a subsequent session with the book will probably be very helpful for visitors who cannot absorb all the information offered by the exhibits.

The museum authorities foresee a need to control the flow of visitors through the exhibition, and they are waiting to see how it survives the rigours of the next few months.

Mary Lindley

Mary Lindley is Assistant Editor of Nature.

Life as a self-perpetuating information system

Genetic Evolution. By Chen Kang Chai. Pp. 341. (University of Chicago: Chicago and London, 1976.) £15.

THE theme of this work is that life is a self-perpetuating information system and that at all levels this undergoes revision or evolution. The author considers, in sequence, the evolution of prebiotic molecules, of genes, symbiosis, the evolution of chromosomes, of biological structure and function and of populations. In spite of protestations otherwise, this cannot be considered a text book; indeed its use as such would be irresponsible. Those without a broad elementary understanding of modern genetics would find this book difficult, and it contains much that should not be uncritically accepted by a genetics undergraduate. It is a hybrid between a collection of essays and a textbook (say, of population genetics); the phenotype of the organism is stimulating and interesting in places; biased and superficial at others. The subjects covered are so broad that response to a particular chapter is likely to be conditioned by previous experience of its subject matter.

Chapter 1 is concerned with the evolution of biological molecules and the genetic apparatus. It is straightforward and recalls the work of Oparin, Calvin and others on the evolution of organic molecules from inorganic precursors. The next chapter deals with the evolution of genes and perpetuates the belief that the rate of amino acid substitution (and thus the rate of change of DNA) is constant for particular proteins. This is no longer upheld by data for cytochrome C and haemoglobin. The α and β chains of haemoglobin have evolved at different rates, and the same chain has evolved at different rates during vertebrate history.

The third and fourth chapters deal with symbiosis as a precursor to sexual reproduction and with the evolution of eukaryote chromosomes respectively. These two chapters are absorbing but one is left with the uncomfortable feeling that the evidence for each thesis is tenuous to the extreme. When dealing with the evolution of structure and function the book is more convincing and the (freely admitted) sin here is one of omission. The remaining chapters largely deal with population genetics with the material arranged to suit the underlying theme of the book. Here, the discussion of genetic load seems uncritical as is the examination of the current controversy between the neo-Darwinists and those subscribing to neutral mutation theory. The author obviously believes that most variability is of selective importance; yet as with data on the constancy of the rate of amino acid substitution, he does not stress alternative arguments.

If one accepts this book's F_1 status (and protects the innocent), it is interesting and enjoyable. With appropriate supplementary material it should form the basis of many lively honours level seminars and discussions.

J. A. Bishop

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Chemical lasers

Handbook of Chemical Lasers. Edited by R. W. F. Gross and J. F. Bott. (Wiley Interscience: New York and London, December, 1976.) Pp. x+744. £31; $49.

THE preface of this volume describes how a meeting was convened in California in September 1964 to discuss the possibility of nonequilibrium chemical excitation and pumping of lasers, and how by the close of the conference a young graduate student was able to claim that he had demonstrated the first photo dissociation laser. In that experiment excited iodine atoms were formed by the photoinducted rupture of alkyl iodine bonds; this work led naturally to the demonstration of chemically-pumped emission in photolytically-initiated hydrogen–chlorine explosions by the same workers (Kasper and Pimentel) in 1965. It is instructive to speculate why so few of the resultant publications in this interdisciplinary field have emanated from the United Kingdom or indeed why relatively few of the twenty authoritative contributors to this handbook work in western European laboratories.

The editors do not claim to have created a basic text book, but rather an up-to-date compendium of relevant (high gain) laser physics, the gas dynamics of reactive flows and the chemical kinetics of nonequilibrium reactions. Pulsed and continuous hydrogen–halide lasers, the carbon monoxide laser, high power photochemical iodine lasers and metal-atom oxidation lasers are treated in detail; between 40 and 240 references per chapter ensure that most of the published journal and report literature between 1967 and late 1974 is well represented. Problems of numerical (gain) modelling certainly merited (and received) a chapter of their own; rather unexpectedly the treatment of optical resonators also extended to some 120 pages. Like many of the other contributions, however, this chapter (by Chester and Chodzko) was a delight to read; their treatment of stable and unstable resonator theory, and the complications introduced by the laser-gain medium in matching theory to experiment, is a jewel of conciseness. The book is well produced and edited, a 'must' for libraries, but rather expensive for the individual research worker.

Ian Spalding

Ian Spalding works on laser applications at the UKAEA Culham Laboratory, Abingdon, UK.

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Explanations in criminology

Behaviour and Misbehaviour: Explanations and Non-Explanations. By Nigel Walker. Pp. ix+154 (Blackwell: Oxford, 1977.) £5.

MOST new disciplines go through a period of introspection in which they examine the philosophical status of their theories and the adequacy of their methods. Criminology has not yet emerged from this stage, and in *Behaviour and Misbehaviour* Professor Walker examines the nature of explanation in that subject.

He distinguishes between "necessity explanations" and "possibility explanations". The former are used to explain regular events such as the daily rising of the Sun or the expansion of a gas on heating, the latter to explain exceptional or unique events. If an iceberg appears as far south as Rockall, we do not ask why it was *necessarily* there but what made it *possible* for it to be there: the answer required is a narrative involving such factors as a particularly hard winter in Greenland, the prevailing ocean currents, and the existence of strong north-westerly winds. Professor Walker calls the former type of explanation "scientific" and the latter "narrative". He then argues that because much criminal behaviour is exceptional, criminologists may have to be satisfied with narrative rather than scientific explanations. He is probably right: even rates of crime, as well as individual crimes, are determined by many interacting features some of which may be unique to a given society. But it is not clear that the difference between theories in the physical and social sciences is entirely captured by the distinction between "narrative" and "scientific" explanations: as Professor Walker acknowledges, even the physical scientist usually has to fall back on the narrative explanation to account for individual events such as the failure of a bridge.

A further distinction drawn by Professor Walker is that between explanation by analogy and explanation in terms of mechanisms. The difference is by no means clear-cut: an explanation by analogy may in fact be implicitly proposing a mechanism the details of which are not fully worked out. When Harvey had the insight that the heart was a pump was he suggesting an analogy or a mechanism? Exception can be taken to Walker's contention that explaining human behaviour by computer simulation is only giving an analogy: a computer program can represent the mechanisms underlying behaviour in just the same way as a set of equations can represent the mechanisms underlying the behaviour of a physical system.

Some of Professor Walker's omissions are suprising: in discussing scientific explanations he says little about formal rigour, simplicity, elegance or generality. He does, however, rightly castigate his fellow criminologists for the idiocy of some of their disputations such as the controversy over whether a given phenomenon can only have a single explanation and the dispute about the range of phenomena that fall within the province of criminology. He also gives short shrift to the arguments of Peters, Winch and others that human behaviour can never be understood in terms of causes.

I have two complaints about the book. For its exorbitant price, one might reasonably have expected the publishers to have hired a more conscientious proof reader. The second complaint is more unusual: although the book raises interesting issues, it is too short to do justice to the complexity of its subject matter, and it should have been several times as long.

Stuart Sutherland

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Enzyme kinetics

Enzyme Kinetics. By D. V. Roberts. Pp. 326. (Cambridge University: Cambridge, London, New York and Melbourne, 1977.) Hardback £15; paperback £5.50.

THIS book deals with the use of mathematical expressions to describe the behaviour of enzymes and enzyme systems. Although the author works through the derivations of many of the equations that he presents, some familiarity with the use of determinants and the calculus is necessary if the reader is to follow the arguments used, and the book could not be recommend to mathematically weak students. For those who are not frightened-off by the mathematical treatments used, the author's comparison between the use of determinants and the schematic King–Altman procedure for deriving initial-rate equations is valuable, as is his treatment of the use of the Laplace–Carson operator method for solving the differential equations describing pre-steady-state behaviour. At times the derivation of equations seems to take precedence over consideration of the phenomena that they attempt to describe. In the chapter on allosteric proteins, for example, it is unfortunate that the author does not give more emphasis to the predictions made by the different models that he considers and to ways in which they may be distinguished.

The coverage given to different aspects of enzyme kinetics varies greatly and some topics, such as the effects of *p*H and temperature on enzyme activity, are given rather shallow treatments. In some places the analyses presented are misleading. The section on the use of product inhibition as an aid to defining kinetic mechanism, for example, fails to consider the effects of abortive ternary complex formation in random mechanisms and thus predicts an inhibition pattern for the rapid-equilibrium case which is in fact rare. In addition failure to distinguish clearly between the exclusive and non-exclusive cases of the concerted model of co-operativity leads to confusion in the analysis of the curve shapes predicted by this system. The author's differentiation between the steady-state and rapid equilibrium treatments of kinetic mechanisms is also confused and this is particularly evident in his treatment of non-competitive inhibition.

The main strengths of this book lie in its treatments of the kinetics of coupled enzyme systems and of computer simulation methods. This latter chapter, which considers the use of both analogue and digital computers, is particularly valuable since this important topic is usually ignored in books of this type. The author's enthusiastic treatment should capture the interest of many students and although the section on digital methods would not be sufficient to enable the reader to write his own simulation program, it provides an excellent introduction to the terminology and methods used. The sections on the use of analogue computers define the components used and give sample programs for simple kinetic systems. These programs should be sufficient to stimulate many enzymologists with access to an analogue computer to take this approach further.

K. F. Tipton

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Pipet stand. Variable Volumetrics. The D-2 pipet stand is made of transparent black plastic. It has stations for holding 3 Finnpipettes in the 1–1,000 μl range and 1 Finnipipette in the range 1,000–5,000 μl. It has good storage capacity for tips. Dimensions: 11 inch by 7½ inch by 6 inch.

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Microstirrer. T.E.M. The Temtron will continuously stir micro quantities of aqueous chemical compounds. Previously this has not been possible under certain restrictive operating conditions. A method is now available allowing effective stirring, also addition of further preparations to existing compounds without ever handling the cuvette or disturbing the sensitive, fully enclosed detector system of the analyser. Stirring rate is continuously variable from 0–30 Hz. An 11 mm × 11 mm × 5 mm energiser capsule is placed under the cuvette and drives a Teflon-coated stir bar magnet. Cuvettes (e.g. 1 ml) having internal physical shape restrictions as small as 3 mm × 10 mm opening, normally impossible to stir, can be accommodated. The microstirrer does not interfere with the light sensing detectors.

Circle No. 43 on Reader Enquiry Coupon

Automatic photometer. Anaspec. The Biophotometer is useful in microbiology, bacterial research, the control of sulphamides and antibiotics, cancer research, oceanography and biology. Monochromatic light is presented successively to six separate cultures, each contained in an agitated temperature controlled environment. The measured optical transmisison is then monitored on a six point recorder for the growth period of the culture.

Circle No. 46 on Reader Enquiry Coupon

Shock chambers. Tenney Engineering Inc. Controlled by the Tenny DigiTenn microprocessor programmer, the multi-compartment cabinets perform all operations automatically once the desired sequence has been keyed into DigiTenn. Thermal shock chambers have two compartments, one at −65 °C, the other at 204 °C. Test specimen is transferred between thermal spaces, alternately shocking the item with extremes of heat and cold. In a typical DigiTenn controlled model, seven event channels determine temperatures in each chamber, time of work transfer from each chamber individually. time interval for temperature recovery, and number of transfers completed before automatic shutdown.

Circle No. 45 on Reader Enquiry Coupon

Nanotech vacuum coater

Vacuum coaters. Nanotech. The Semprep 2 sputter coater unit has automatic and manual operations. Facilities include specimen etching, water cooling of both specimen and and target, triode systems to minimise electron heating effects and controlled argon flushing and venting. Variable sputtering voltage up to 3 kV at current levels up to 150 mA; integral two stage 3 $\mathrm{m^3\,h^{-1}}$ rotary vacuum pump with pirani gauging, complete metal pipework and viton seals to reduce contamination levels.

Circle No. 46 on Reader Enquiry Coupon

Preparative ultracentrifuges. MSE. The Prepspin 65 and Prepspin 75 feature a new type of speed selector, a water shut-off valve to conserve water, a new semi-hermetic refrigeration unit, a brush warning indicator to indicate when brushes need attention or renewing, automatic shut down of gas ballast, a new design of oil mist filter, cushion start, and a completely redesigned control panel.

Circle No. 47 on Reader Enquiry Coupon

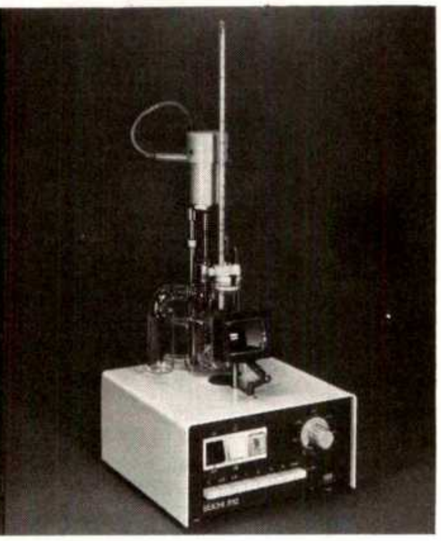
Tweezers. Welwyn Tool. Suitable for accurate handling of small, delicate components in the engineering, electronics, optical, chemical and other specialist industries, Granit tweezers have a smooth satin finish and are available with a variety of point configurations, including long and short fine, super fine, extra long, oblique, angled and curved. Length from 4 to 5 inch. Stainless steel and non-magnetic versions are available, the latter particularly suitable for handling small ferrous components. The non-magnetic models are resistant to most corrosive chemicals.

Circle No. 48 on Reader Enquiry Coupon

High speed servo recorder. J. J. Lloyd. The CR600 is a new two pen flat bed recorder combining fast writing speed with high accuracy. The recorder, in corrosion-resistant, brushed stainless steel case is suitable for vertical or horizontal use and is provided with chart take up facilities for both 'Z fold' and roll chart paper. Electronic limit switches deccelerate the pens at the end of their travel reducing wear often associated with high speed servo recorders. The advance specification includes an 18-speed reversible electronic chart drive ranging from 0.01 mm $\mathrm{min^{-1}}$ to 10 mm $\mathrm{s^{-1}}$, a sensitivity range from 5 μV $\mathrm{mm^{-1}}$ to 0.5 mV $\mathrm{mm^{-1}}$ by stepped attenuator with a vernier span control to provide complete range overlap. Special facilities include an internal voltage reference for spot calibration checks at any sensitivity setting, a 1,000% precision calibrated suppressed zero, remote controlled chart drive, pen lift and event marker. The chart width is 250 mm and writing speed exceeds 600 mm $\mathrm{s^{-1}}$. Options include retransmitting potentiometers on both channels and an integrator.

Circle No. 49 on Reader Enquiry Coupon

Buchi nitrogen distillation unit



Buchi melting point apparatus

Distillation unit for nitrogen determination. Büchi. The 320 is built into a housing with the glass parts protected from breakage, has built in storage tanks (5 l) for sodium hydroxide solution and for distilled water, the residue can be sucked out of the vial after distillation and the digestion tube also serves as distillation vial; it is, therefore, unnecessary to transfer the sample.

Circle No. 50 on Reader Enquiry Coupon

Melting point determination apparatus. Büchi. The 510 has an immersion depth of samples and thermometer of 40 mm regardless of the oil temperature. By this means, the 510 corresponds with the method of European Pharmacopoeia (capillary method). The new model also permits the use of Pharmacopoe-Thermometers calibrated at an immersion depth of 40 mm, with a subdivision of 1/5 °C.

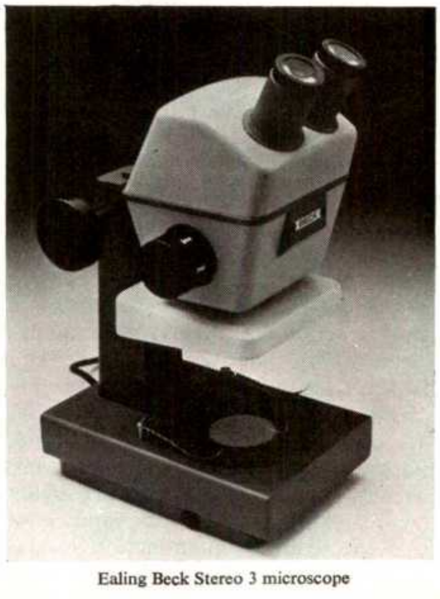
Circle No. 51 on Reader Enquiry Coupon

Stereo 3 microscope. Ealing Beck. Binocular optical design with a 10° convergence angle provides a stereoscopic effect suitable for inspecting such objects as laminated substrates in integrated circuits. Three optical systems of different magnification are included in the microscope head. The objective lenses for each magnification are mounted in a rotating turret which can be operated from either side of the microscope head. A parfocal system ensures that the object being viewed remains in focus at all magnifications. A useful feature is the long working clearance between the object being viewed and the microscope head. Depending upon magnification, the working clearance on the Stereo 3 can vary between 100 mm and 150 mm and is ideal for manipulative operations such as the bonding of miniaturised circuits.

Circle No. 52 on Reader Enquiry Coupon

Cell harvester with pre-programmable four wash sequence. Dynatech. For techniques where it is desirable to harvest cell cultures with more than one wash fluid, the Multiwash has been designed to deliver, in sequence, up to 4 wash fluids, including TCA, completely automatically to a row of 12 wells of a Microtiter plate, while simultaneously aspirating the contents of each well onto a fibre glass filter for subsequent analysis. Simple push button control enables selection of any combination of wash fluid; and wash times for each fluid may be independently varied between 2 and 60 s by pre-programmable time controls. Water vacuum may be sufficient for operation but a suitable vacuum pump is an option, if mains pressure is insufficient. Also standard inter-changeable wash/aspirate heads to facilitate harvesting from standard test tube racks and also have the facitity to manufacture special heads to customer specification, if desired.

Circle No. 53 on Reader Enquiry Coupon



Ealing Beck Stereo 3 microscope

Tubular filtration system. Millipore. The Steriflo system is designed to handle product volumes up to several hundred litres, and is ideal for applications such as: final filtration on filling machines, batch filtration and particle removal filtration. The system has a unique tubular membrane filter which combines the simplicity of disk filters with the ruggedness of conventional cartridges. Composed of cellulose ester membranes, this new filter is a hollow capless tube sheated internally by a layer of high wet-strength cellulose and externally by a monolithic polyethylene web. Each end of the tube is permanently edge-sealed with a dip coating of urethane. Product flow through the filter is from inside (upstream), out (downstream), providing high resistance to operating stresses. Where extended filter life is required, tubular Steriflo prefilter is also available that can be concentrically installed in the same housing. Also composed of cellulose esters, this tubular prefilter is free of asbestos and glass fibres, making them suitable for parental processing.

Circle No. 54 on Reader Enquiry Coupon

Radiation alarm monitor. Philips. The PW 4544/07 alarm monitor is designed to ensure a safe environment in X-ray analytical laboratories. It provides continuous confirmation that radiation levels are clearly within pre-set safety limits and that rooms containing X-ray producing equipment may be entered safely. The PW 4544/07 is designed for detection of 'soft' X-rays. The monitor is supplied with an external probe containing the end-window tube ZP 1600 (18507) which is fitted with a $1\ \text{cm}^2$ window to cover the range 0.02–$200\ \text{mR h}^{-1}$ (50 keV). The required alarm level indicated by red light and buzzer may be preset set at any point in the range.

Circle No. 55 on Reader Enquiry Coupon

Ultra-low-temperature cryogenerator. Philips. A compact cryogenerator, supplying cold in the 20 K and 80 K ranges simultaneously. The standard type K-20 cryogenerator, is particularly suitable for cryopumping, in high-vacuum or ultra-high-vacuum systems. The cryogenic pump is probably the preferred device for applications like space simulators, linear accelerators and thin-film deposition. The unit can be incorporated into systems designed for these purposes, including industrial coaters. Other application fields include gas liquefaction systems and low-temperature refrigeration systems.

The educational version of the K-20 provides additional measuring devices which allow students to carry out complete evaluations and assessments of various cryogenic phenomena.

Circle No. 56 on Reader Enquiry Coupon

nature

14 July 1977

Million-dollar problem—billion-dollar solution?

THE OECD report on the transport of air pollutants which Dr Barnes summarises on pages 92–93 is bound to resurrect some political problems that have been held just below the surface whilst the report was being prepared. The most important is likely to be the question of whether the United Kingdom should assume any responsibility for the effects of acid rainfall in Norway.

In the past thirty years the greatly increased use of tall smoke stacks by industry has been a blessing to those who live in industrial areas and who are no longer bathed in the acidic fumes of sulphur dioxide and sulphates. But the disagreeable material is now not simply dispersed over a wider area surrounding the smoke stack; much of it resides for a long time in the mixing layer that extends to a height of about 1,200 metres and which has been described as a travelling garbage can. The material is eventually either dry-deposited or brought down to the earth as acidic precipitation.

The OECD study reports sulphur deposition levels in rural areas in 1974 and attempts, by an understanding of prevailing winds and precipitation, to estimate how much of the material comes from the country in question, and how much from elsewhere. A table attempts to summarise the export–import budget for most European countries (including estimates for Eastern Europe). It is open to misinterpretation by politicians who do not appreciate the very large error bars (at least ± 50%) that must be applied to the data and the relatively short period of reporting (one year).

Even so, some interesting figures emerge. In many countries exports and imports of sulphur roughly balance, but Austria, Finland, Norway and Sweden and Switzerland each involuntarily import more than twice what they export. Denmark and the United Kingdom, on the other hand, export much more sulphur than they import.

The United Kingdom is the largest sulphur-emitter in Europe, putting 1.8 million tonnes into the atmosphere in 1974 (remember, however, that the figures must not be read too precisely). Less than half of this comes back to earth in the UK—most of the rest of Europe getting a dose of British sulphur. In the case of, say, West Germany, the dose is relatively small compared with what West Germany administers to herself. But Norway, where the clouds that pass over Britain often deposit their rain, receives roughly a quarter of her total sulphur deposit from British sources, double the contribution she makes from her own smokestacks.

This in itself by no means makes Norway into an exceptionally polluted part of the world—wet deposition of sulphates in southern Norway runs at about the same level as in much of the rest of Europe. But a lot of this sulphate resides in snow for several months and is the first to melt when temperatures rise in spring. The acid flush down rivers (pH 3 to 4) then kills fish, doing damage to the value of around $1 million. Sulphur also does other damage, of course, through corrosion and possibly by affecting agricultural productivity (though this is not yet well understood) but such damage is probably common to many countries whereas the fish kill problem is mainly relevant to Norway. It has also to be said that Norway worries about its declining environment in much the way the United Kingdom worries about its declining economy.

But now we know a little better what happens to sulphur, what is going to happen? Norway, which in the past has arranged for journalists to be flown to see the ravages caused by British pollution, will presumably go on the offensive again and demand better removal of SO_2 and sulphates at source. The United Kingdom will say that the study represents only one year and is of necessity so inexact that policy decisions cannot be based on the report.

The bleak fact is that this diseconomy, on a scale of ten million dollars at most, can only be countered by corrective action on a Europe-wide basis costing at least one thousand million dollars, maybe ten times as much. There is, of course, a solution of sorts, and that is for Britain to supply Norway with limestone to be added to lakes during the acid flush. No-one is yet sure that this will save the fish without some other side effect emerging. And of course it is of the nature of a technological fix—a now much reviled practice; but so, for that matter, would be anything attached at vast cost to chimneys. Nonetheless, the Norwegian government would be foolish to reject such a proposal out of hand—as seems quite likely. An insistence on removing pollution at source calls for so much investment and will generate so much international ill-will that any more flexible solution must first be considered. □

Twelve months after Séveso

A YEAR AGO last Sunday, Séveso 'happened'. A trichlorophenol reactor at the Icmesa chemical plant there spewed its contents over the surrounding area. They included about 1 kg of a highly toxic by-product, 2,3,7,8-tetrachlorodibenzo-paradioxin, known as dioxin. Controversy has raged ever since. It has involved both the plant's Swiss owners (Givaudan, a subsidiary of Hoffman-La Roche) and the Italian authorities; the scientific, legal and other aspects of the disaster; and, above all, the many people affected directly or indirectly by the dioxin. Séveso has received probably the most extensive media coverage ever accorded to the site of an industrial accident, and is a watchword for pollution.

Amidst all this two facts about the disaster are irrefutable, their consequences disastrous. The first was the venting of the reactor discharge directly to the atmosphere, an obvious fault of design. The second was the two-week delay in evacuating residents from the area, responsibility for which cannot with certainty be established but is part of the deliberations of the Italian court investigating the accident. For the people exposed to the dioxin, the health picture shows 106 children with the skin disease chloracne, but no evidence of immunological damage; no evidence of malformation attributable to the dioxin in the foetuses legally aborted from Séveso women exposed to the chemical, and no evidence of chromosomal damage in these foetuses; and a number of children born with abnormalities in the Séveso region which is reported to be no greater than the statistical norm for the area.

The plan to monitor the health of everyone exposed to the dioxin, though medically and politically necessary, will not be a true epidemiological survey of 'dioxin risk'. A more selective survey of categories of 'high, low and non-risk' groups is under review and will yield results more rapidly. There are fewer grounds for optimism over the question of decontamination policy for the removal of the dioxin. The section of Séveso which housed most of the evacuated residents has been decontaminated by the plant's owners and pronounced safe for habitation. But the methods to be adopted for the remainder of the contamination area are still disputed.

What are the wider implications of Séveso? The issue of reform of the abortion laws in Italy is one. Another concerns one of the proposals of the 1976 World Health Assembly which advocated that WHO collate information on birth defects in the populations of member countries; the collation is now more urgent than ever. The lessons for the general public, the chemical industry and governments are obvious. There are dangers involved in the operation of chemical plants. And the cost of disasters in the industry is great. Governments need to keep their pollution control measures under constant review. The fact remains, however, that it takes a major disaster to make things happen; and that has been of little comfort to the people of Séveso over the past 12 months. □

Rational containment on recombinant DNA

The US National Institutes of Health has recommended that all recombinant DNA experiments, involving any animal virus, should be subject to the highest containment conditions, even though other work with the intact virus may require less stringent conditions. Lennart Philipson, of the Department of Microbiology, Uppsala University, Sweden, and Pierre Tiollais, of the Institut Pasteur, Paris, put forward the case for categorising recombinant DNA experiments involving animal viruses more rationally.

THE development of molecular genetics has depended largely on the detailed analysis of bacterial viruses, the bacteriophages, and their use to transfer bacterial genes between bacteria which do not normally recombine. Animal viruses now promise to provide a similarly detailed insight into the molecular mechanisms of gene expression in animal cells, which will undoubtedly contribute to an understanding of man and his domestic animals both in health and disease and, it is hoped, contribute to the development of medical and veterinary science.

Restriction enzyme mapping of the viral genome enables regions of interest to be excised and analysed. In many cases the full development of this work requires the use of recombinant DNA techniques to purify and clone the resulting portions of the viral genome. Animal viruses can also be used as vectors for introducing other eukaryote DNA into animal cells in which its expression may be studied. At present, however, the guidelines on recombinant DNA research laid down by the US National Institutes of Health (NIH) require almost all recombinant DNA experiments involving animal viruses to be carried out under the highest containment conditions. In practice these requirements preclude most experiments.

Animal viruses as a class are unfortunately regarded by the general public and the uninformed scientific community as rather mysterious entities which cause pandemics, epidemics and isolated cases of dreadful infectious diseases against which there is no remedy. Scientists themselves have coloured this picture by suggesting that viruses may cause cancer in man, implying to the layman that cancer is an infectious disease. In reality, animal viruses are extremely diverse; some are known to be highly infectious and pathogenic. Others seem to be harmless. It should therefore be mandatory to discriminate between different viruses according to their pathogenicity to man and other mammals when formulating guidelines for the use of viral genomes in *in vitro* recombinant DNA research.

The conjectural hazards envisaged arise from the (hypothetical) risk that virus genomes, when introduced into bacteria or when used as vectors for introducing DNA into mammalian cells, may give rise to "new infectious entities" or may transfer their pathogenic or tumour-producing capacity from the new host back to man or other mammals. Both types of risk depend on chains of events with low probabilities.

Against these arguments it should be considered, first, that the transfer of all or part of the viral genome to bacterial cells may not be an entirely new event. For millions of years prokaryotic organisms have been exposed to eukaryotic DNA and several microorganisms have efficient systems of transferring DNA over recombinant barriers. Second, the development of a 'new infectious entity' would probably require expression of the viral genes in the prokaryotic cell. This must have a low probability since most genes from eukaryotes and viruses, which have been transferred up till now, are not faithfully expressed as RNA or protein in bacteria[1-4]. Even if the inserted viral genes are expressed the resulting risk would not be greater than that from production of viral proteins in cell cultures, since only fragments of the viral genomes will be inserted in the experiments proposed.

The conjectural hazards may therefore be confined to the transfer of viral nucleic acids from the modified bacterium to an animal or human host. This would require a chain of events, each of low probability: the bacterium containing hybrid DNA must first escape from the laboratory and then establish itself in a new host. The viral nucleic acid must then enter the cells of the new host, and there express its tumorigenic or other harmful properties. Even without considering containment we are probably discussing probabilities in the range 10^{-20}–10^{-28} for the transfer of harmful genes into the new host.

Against this background it is obviously necessary to prohibit experiments with viruses such as Lassa fever virus or Marburg virus, which are considered to be high risk viruses in diagnostic laboratories; but it is not clear that viruses classified as low risk, such as adeno, SV40 and polyoma viruses, need such high containment. SV40 virus has already been injected accidentally together with polio vaccine into millions of human subjects without any registered harmful effects[5,6], although antibodies against SV40 appear in the serum. Some adenoviruses infect humans readily and about 50–80% of the population develop antibodies to adenovirus types 1 and 2 from the age of 5–10 years[7]. Even the adeno–SV40 hybrid viruses which were originally isolated when adenovirus vaccines were developed in monkey kidney cells have been introduced into large groups of military recruits as an inactivated or live vaccine without any detrimental effects[8,9]. Careful studies have failed to find an association between adenovirus transcripts, T antigens or other adenovirus products with tumours in humans[10,11].

Normal biochemical work with adeno and SV40 viruses is considered to fall into the low risk category under guidelines published by the National Cancer Institute and those from the Center for Disease Control, Atlanta, Georgia. These regulations allow the use of large amounts of virus. Milligram quantities of viral DNA are handled regularly by investigators and technical personnel in several biochemical laboratories. Provided that expression of virus DNA is prohibited in the bacterial cell, which is likely (see above), it must be more risky for personnel to handle viral DNA than to insert it in a bacterial vector. The NIH guidelines request P4 and EK2 conditions to handle partial or intact viral DNA in a prokaryotic host although as we have pointed out the risks envisaged are comparable to or lower than those encountered when working with intact virions. It is possible to claim that virions present a higher risk since as long as the protein coat is present the virion may possibly escape from the laboratory and infect humans. Thus, there seems to be a disproportionately high containment requirement for work with animal virus genomes in bacteria.

It is also difficult to understand the containment requirements for work with eukaryotic vectors. In the case of SV40 it has been established that SV40 DNA will form hybrids with cell DNA when cells are infected at high multiplicity[12,13]. Such experiments are in essence shotgun experiments, but since no *in vitro* recombination is involved it is considered a low risk experiment requiring no containment facilities. If a similar experiment is carried out *in vitro* between DNA from the same cell and SV40 virus DNA (probably with lower efficiency), it is labelled as P4 according to the NIH guidelines. Insertion of SV40 sequences into adenovirus DNA also occurs frequently when the two are cultivated together, as exemplified by the adeno–SV40 hybrid viruses[14,15] and new hybrids recently developed (J. Sambrook and G. Fey, personal communication). To take advantage of the natural recombination between SV40 and adenoviruses requires only a moderate risk containment facility, but the NIH guidelines absolutely forbid these experiments using recombinant DNA techniques. Here there is also a distinct discrepancy between the guidelines and other regulations for the study of animal viruses.

Most of the confusion is probably due to the fact that natural and artificial recombinant DNA reserch has not been considered as a whole. Only the new *in vitro* recombinant DNA technique has formed the basis for developing the NIH guidelines, although guidelines for work with dangerous microorganisms have been in existence for a long time. Consistent rules for all recombinant DNA research are therefore needed.

A good definition of recombinant DNA research might be that suggested by the Standing Advisory Committee on Recombinant DNA of the European Molecular Biology Organisation (EMBO): recombination of DNA molecules of different biological origin by any methods that overcome generally recognised natural barriers to mating, infection and recombination, to yield molecules that can be propagated in some host cells and the subsequent studies of such recombinant DNA molecules. *In vitro* recombinant DNA research is only a subsection of this definition. Within the broader meaning of the term there are several experiments now carried out in low risk containment according to generally accepted guidelines, which must be analysed and compared with the *in vitro* techniques before any regulatory guidelines are issued. It is, for example, possible to transfer the genome of several animal viruses into the capsid of unrelated viruses[16–19], and thereby increase the host range of the viral genes. It is also possible to fuse animal cells, which may involve undesirable transfer of viral genomes to new hosts[20,21]. Many of these experiments involve conjectural hazards comparable to those implied by the *in vitro* recombinant DNA technique.

But in these cases the experiments also probably carry only a low risk if they involve low risk viruses, since similar events probably occur in nature. Therefore we would like to propose that the entire area of recombinant DNA research should be re-evaluated. Experiments which are considered to involve real and proven risks should be prohibited at present. The transfer, by any method, of high risk viral genomes, potent bacterial toxins and antibiotic resistance to gonococci and streptococci fall into this category. Conjectural hazards which may or may not become real should rapidly be evaluated under secure conditions. Meanwhile *in vitro* recombinant DNA research can probably proceed in several areas including the transfer of low risk viral genomes to prokaryotic cells and the development of eukaryotic vectors from low risk viral genomes.

In closing, it should be emphasised that both mutagenisation and deletion of a multitude of genes in the laboratory have, to the best of our knowledge, never provided a selective advantage for an organism in the natural environment. Some genetic manipulation with plant cells may be an exception. It is necessary for the opponents of recombinant DNA research to provide examples of such selective advantage otherwise this debate will focus more on faith than science. □

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Sulphur deposit account

R. A. Barnes looks at an OECD study on the transport of air pollutants over long distances

THE Final Report of the Organisation for Economic Cooperation and Development (OECD) study on the long range transport of air pollutants within north-west Europe was derestricted last week. It has its origins in the first European network of sites to monitor the chemical composition of precipitation which was established in Scandinavia in 1947. In the 1950s this was extended to other countries and became known as the European Air Chemistry Network. A first detailed analysis of the data, performed by Odén in 1968, showed that an area of highly acid precipitation (pH 3–4) located in the industrial heart of Europe was expanding from year to year. This finding was paralleled by the observation that rivers and lakes in southern Scandinavia were also becoming more acidic and so it was decided that a more detailed study of the origins of the acidity was required.

The Scandinavians in particular have expressed great concern that the increasing acidification of inland waters was causing the observed fall in fish populations and was also reducing forestry production. In their opinion, the sources of acid precipitation were sulphur dioxide emissions from the heavily industrialised countries of north-west Europe, particularly those from the UK. This latter view was based on the assumption that south-west winds prevailed and that the UK policy of making major industrial emissions from tall chimneys (the 'tall-stack' policy) to reduce nearby surface concentration—which affects about half the total UK emission—is particularly effective in encouraging the transport of pollutants over long distances.

The OECD programme began in 1972 with the aim of determining the relative importance of local and distant sources of sulphur compounds within European countries, special attention being paid to the question of precipitation acidity. Initially ten countries agreed to participate actively—Austria, Denmark, the Federal Republic of Germany, Finland, France, the Netherlands, Norway, Sweden, Switzerland and the United Kingdom—and in 1974 Belgium also joined. Although several surveys have already been made of air pollution fluxes and depositions within certain parts of Europe, the OECD study is the first comprehensive investigation of air pollution movement on a continental scale. Data analysis and interpretation, together with preparation of the final report, has been the responsibilty of the Norwegian Institute for Air Research.

The research programme consisted of observations based primarily on a network of sampling stations, and predictive modelling. In all, 76 ground level sites, sampling on a daily basis, were in operation at one time or another—8 in the UK and 23 in Norway. They were located, with moderate success, to represent rural conditions within a geographic region. Mean sulphur dioxide and sulphate aerosol concentrations observed at these sites ranged from about 20 μg m^{-3} and 10 μg m^{-3} respectively, close to the major source regions, to about 2 μg m^{-3} and 0.5 μg m^{-3} respectively in the remote areas of northern and western Europe. The higher of the sulphur dioxide figures would be approximately a factor of 5 less than in the source areas themselves, whereas the longer lived and slowly formed sulphate species would be more or less the same. The sulphate in precipitation data show a similar geographical distribution to the air concentrations, with values ranging from 5 to less than 1 mg l^{-1}. In some areas (for example south-west Scandinavia and Switzerland), however, where locally increased precipitation produces greater deposition than in surrounding districts, the pattern is modified. Strong acid in precipitation shows a distribution similar to that of sulphate but with notable differences in some areas. It seems to be due to both sulphuric and nitric acids in proportions of between 3 : 1 and 1 : 1.

Sector analysis

A sector analysis was performed on the surface measurements. Using 850 mbar (1,200–1,500 m altitude) trajectories they were allocated to six directional arcs and a mean value was calculated. The results show that high mean concentrations and/or depositions were not randomly oriented, but were directed towards the main areas of emission. When these emitting areas lie to the west of the receptor, concentrations are relatively low but aggregate depositions high. The low concentrations are probably a consequence of the higher wind speed and fewer stagnant, stable, conditions in westerly regimes, while the higher depositions would be related to the greater frequency of precipitation. In most parts of western Europe, higher concentrations are usually found in sectors directed towards the south and south-east, although these do not necessarily result in particularly high annual deposition totals. Winds from such directions are generally associated with a higher frequency of stagnant and stable conditions over source areas which would lead to the high concentrations, and with limited convective or frontal rainfall, wet deposition would tend to be limited. In south-west Norway, however, orographic precipitation in southerly and south-easterly winds, coupled with high sulphate concentrations, produces annual depositions in these sections comparable to those experienced in the westerly winds where precipitation is more frequent and plentiful.

Unfortunately, the sector analysis does not, in general, give a clear indication of which sources give rise to what fraction of the concentrations in a given sector. Because of its geographic position and relative isolation, however, an estimate can be made of the mean contribution of emissions in the British Isles to deposition in Norway and the Netherlands. In addition to the average 0.8 mg SO_4 l^{-1} and 25 μEq l^{-1} strong acid background in rainfall from Atlantic air, to which sources in North America seem to contribute, emissions in the UK and Ireland give rise to 1.0 mg SO_4 l^{-1} and 20 μEq l^{-1} strong acid. Mean concentrations at Norwegian sites due to other emission areas range up to 8.7 mg SO_4 l^{-1} and 140 μEq l^{-1} strong acid.

Several of the participating countries used instrumented aircraft to obtain data on above-surface concentrations of air pollutants. Flights were usually made under specific meteorological conditions and never at night or in cloud. The results confirm that under these conditions, sulphur compounds are normally transported in the lowest 2 km of the atmosphere, generally with a peak concentration a few hundred metres above ground level and a progressive decline above that height. The average mixing height under flight conditions was found to be 1,200–1,300 m, although individual vertical profiles varied considerably. The flights also showed that under certain conditions distinct plumes of pollutants existed several hundred kilometres downwind of major source areas.

Reference has frequently been made to 'episodes', when substantial amounts of wet sulphate deposition occur over a period of less than a day, without any attempt at a formal definition.

R. A. Barnes is at the Central Unit on Environmental Pollution in the UK Department of the Environment

Since such occasions, on which a substantial amount of the total annual wet deposition at a site may occur on one day, are likely to be of particular ecological significance, a working definition has been formulated: episode-days at a site are those with the highest wet depositions which, when summed, make up 30% of the annual total. An area is 'highly episodic' if 30% of its total annual wet deposition occurs on less than 10% of wet days. By these definitions parts of Norway, Finland, Sweden, Switzerland and Scotland are highly episodic with respect to sulphate. Sulphate episodicity has been shown to be relatively independent of precipitation episodicity.

Mathematical modelling was employed to obtain a more complete pattern of concentration and deposition in Europe. Estimates were made for 1974 only, and are unlikely to be the same for other years, especially in the case of wet deposition. One of the primary inputs to the models was detailed sulphur dioxide emission figures for the whole of Europe. For the 11 participating countries, man-made emissions were estimated to be 9 million tonnes as sulphur in 1973. Emissions of sulphur from other European countries were calculated to be 16 Mt in 1973 from much less reliable data. The total emission of approximately 25 Mt is approximately twice that in 1950, although some countries have contributed to the increase much less than others (the UK rise between 1950 and 1973, for example, was about 30%). Natural emissions were calculated to represent less than 10% of the man-made ones over the continent as a whole.

For use in the models, the national sulphur dioxide emission totals were allocated to a 127×127 km grid, using population and industrial statistics and simple seasonal adjustments made to arrive at emission figures representative of individual days. As a first aproximation 25% of the emission in any grid element was assumed to be deposited in that element.

Estimated budget for dry plus wet deposition of sulphur for 1974 (Unit: 10^3 tonnes of sulphur)[1]

Countries	Sulphur received	Sulphur emitted
Austria	300	221
Belgium	200	499
Denmark	100	312
Federal Republic of Germany	1,250	1,964
Finland	400	274
France	1,000	1,616
The Netherlands	150	391
Norway	250	91
Sweden	500	415
Switzerland	100	76
United Kingdom	1,000	2,883[2]
Czechoslovakia, German Democratic Republic, Italy, Poland and other areas	11,000	—

[1] Numbers are rounded to one significant figure and accurate to within about ±50%.

[2] Includes 80×10^3 tonnes of sulphur from Ireland.

Two models

A Lagrangian model was used to estimate the long range transport of sulphur on a continental scale and the so-called 'Trajectory Model' was employed to estimate the effect of any one country's emissions on the concentrations and depositions in any other country. Both functioned by considering emissions and depositions in individual grid squares and air movements according to 850 mbar (1,200–1,500 m) trajectories. Unfortunately, the choice of a coarse grid (which was necessitated by the low precision of emission data and trajectories, together with limited computing facilities) has caused the very steep gradients of sulphur concentration which exist in reality to be smoothed across 127 km^2. The models therefore only give a very general picture of concentration and deposition with a considerable degree of uncertainty on the accuracy of predicted values at any given point. The relatively coarse grid also made it difficult for one of the main objectives of the programme—the effect of local sources on concentrations and depositions—to be achieved.

The model estimates of daily mean sulphur dioxide and sulphate aerosol concentrations do not agree very well with observed data, correlation coefficients ranging from insignificant values to 0.6 and 0.4 to 0.8, respectively. The poorer correlation in the case of the shorter lived species (sulphur dioxide) reflects the greater effect of grid smoothing in this case. The correspondence between observed and calculated annual mean concentrations, however, at about 0.9 in both cases, is much better.

As would be expected, the estimated dry deposition pattern for sulphur in Europe strongly reflects the emission field with a maximum of 10 g m^{-2} y^{-1} in the major source areas and a minimum of about 0.1 g m^{-2} y^{-1} in remote regions. There is a high degree of qualitative agreement and quantitative similarity between the sulphur wet deposition fields estimated by each of the two models, and that prepared from observed data. To a certain extent, this agreement was to be expected, since all three approaches employ the same grid-smoothed precipitation map. The technique used for preparing the grid assumes zero precipitation more than 100 km from the nearest observation over land and 300 km over the sea. Thus, for grid elements in the centre of the North Sea, the precipitation is reckoned to be about 10% of that in eastern and central England. On a fairly arbitrary basis, it has been estimated that, for this reason, the contribution of British emission to sulphur wet deposition in southern Scandinavia has been overestimated by about 20%.

A regression of observed mean sulphate concentrations in precipitation against weighted mean values of calculated aerosol sulphate concentration, used by the Trajectory Model to estimate sulphur wet deposition due to one country in any other country, has shown that a good relationship ($r=0.9$) exists for Norwegian and Finnish data. The results suggest a natural background sulphate concentration of 0.8 mg l^{-1} in precipitation, a figure confirmed by the sector analysis. This regression has been used in the budget calculations for these two countries. A similar regression on data from the remaining countries, however, shows a much wider scatter ($r=0.36$) and an implied background of 2.0 mg l^{-1}. These results are probably a reflection of the influence of local sources in countries other than Norway and Finland, and would result in a great deal of the wet deposition in a given country being unattributed to any particular source. As a compromise, a relationship using all available data ($r=0.73$) and a 'background' of 1.2 mg l^{-1} is employed for all countries except Norway and Finland.

For several reasons, therefore, the Trajectory Model has not proved capable of estimating the dry or wet deposition in any one country due to emission in any other country to an accuracy greater than ±40%. Thus, the corresponding total (dry+wet) deposition figures are not accurate to more than ±50%. However, the estimates of the Trajectory Model do give a useful indication of the extent and magnitude of transboundary transport of sulphur compounds. Both models indicate that, within the area as a whole, about 50% of the sulphur emitted is dry deposited and 30% wet deposited. The balance leaves the region.

The OECD study has provided a first comprehensive insight into the long distance transport of air pollutants on a continental scale. It is far from definitive, but it has confirmed that sulphur compounds travel long distances in the atmosphere over Europe, and that the air quality in any one country is measurably affected, in a very complex fashion, by emissions in another. Much more precise and detailed work is required if the phenomenon is to be fully quantified, the report itself listing a number of facets which require further investigation. These could be taken into account by an Economic Commission for Europe study, at present in a planning stage. □

Five-year stretch

The USA and USSR have agreed to another five years of scientific cooperation. Colin Norman reports from Washington

WHEN former President Richard Nixon travelled to Moscow in May 1972, he came away with a clutch of agreements for Soviet–American scientific exchange programmes which symbolised the new era of detente. Since then, the warmth of detente has been chilled by disagreements over arms control and human rights issues, but the scientific exchange agreements have proceeded apace. Last week, after a cordial meeting between Soviet and American officials in Washington, the agreements were renewed for another five years.

The renewal was negotiated at a high-level meeting of scientific delegations led by Dr Frank Press, President Carter's science adviser, and Academician V. A. Kirillin, Deputy Chairman of the USSR Council of Ministers, and chairman of the State Committee of the USSR Council of Ministers for Science and Technology. Press later described the meeting as "extremely cooperative, upbeat all the way". To underline the political importance attached to the agreements, President Carter met Kirillin for a widely publicised discussion shortly after the ritual signing ceremony.

This outbreak of cordiality in the midst of Soviet–American ill-feeling lends itself to a number of interpretations; the most widely touted is that the Soviets are willing to put aside their differences to continue with exchanges from which so far they have benefited greatly, while the Americans are eager to demonstrate that the Carter Administration's stand on human rights need not jeopardise mutually beneficial arrangements between the two countries. In any case, extension of the agreements was never seriously in doubt.

In preparation for last week's meeting, however, Press commissioned a review of how the exchanges have operated so far, and as a result there will be some changes in the way the arrangements will work in future. The review was carried out by a committee of the National Academy of Sciences, headed by Richard Garwin, a respected physicist who works for IBM. The Garwin panel interviewed scores of scientists who have participated in the exchange programmes, and its central conclusion was that the agreement should be renewed because of "the positive benefits and real interaction evident in some of the projects under the agreement, [and] the intangible and unevaluated (but widely felt) non-substantive benefits commented upon by the participants". Nevertheless, the Garwin panel pinpointed a number of problems.

The science and technology agreement is in fact only one of about a dozen exchange pacts negotiated as a result of Nixon's May 1972 Moscow summit meeting (separate agreements exist for cooperation in space, environment, energy, medicine and other areas), but it is widely regarded as the most important. It established joint programmes in such fields as chemical catalysis, physics, forestry, the application of computers to management, electrometallurgy and science policy. Some 250 American scientists have participated and the cost to the US treasury has been about $13.2 million over the five-year period.

The Garwin panel reported that progress has been decidedly mixed. In the physics programme, for example, "no exchange or substantive interaction has taken place thus far", while as a result of exchanges in the electometallurgy programme, "familiarity with differences in approach and perceptions in solving technical problems in the USSR has stimulated a considerable amount of rethinking of technical approaches in the US".

There have also been a number of problems common to most of the programmes. For example, the Garwin panel noted that although virtually every participant interviewed emphasised the helpfulness and warmth of individual Soviet scientists at the working level, there appear to be "layers of stifling bureaucracy which enforce a rigidity in the interaction which very much diminishes its value to the US and to the USSR". As evidence of such stifling bureaucracy, the panel cites inflexibility in travel arrangements for US scientists visiting the Soviet Union and lack of notification of the time, date, or place of arrival of Soviet visitors to the United States.

But the problems do not lie entirely on the Soviet side of the exchanges. The Garwin panel noted that US delegations are often ill-prepared before they visit the Soviet Union. There is little interaction between participants in different exchange programmes, and American scientists are seldom briefed on Soviet research and development policies and practices.

The Garwin panel recommended that a small, permanent bureaucracy should be established in the United States government to plan and coordinate the exchange programmes and to prepare US scientists for their visits to the Soviet Union. According to one official involved in last week's talks, such a group is likely to be established soon, probably in the National Science Foundation.

A frequent complaint raised in the United States, particularly among conservatives, is that the flow of information from the scientific exchanges has been virtually a one-way street, from West to East. The argument is that since the Soviet Union lags behind the United States in many fields, it is eager to use the exchanges as a conduit for American technology. The Garwin panel did not specifically address that concern, though it noted in its report that participants in the programme frequently point to "significant political and cultural benefits in addition to the relatively few citations of benefits to US science and technology". Such intangible benefits should also be weighed in assessing the overall impacts of the agreements, the panel suggested.

Nevertheless, the Administration is understandably sensitive to charges that the exchange agreements are resulting in a significant drain of technical information from the United States, and according to one official familiar with the programme, greater attention will in future be paid to securing a better balance of information flow throughout the programme.

Press said that some of the concerns raised by the Garwin panel were brought up during the negotiations last week, and the Soviet participants were willing to discuss ways of smoothing future arrangements. At one point, Kirillin said that if problems arise, he and Press would communicate directly by telephone to iron them out. Asked whether human rights problems were mentioned during the talks, Press said that they were not.

For their part, the Soviets seem anxious to press on with the exchanges, while the Carter Administration seems to feel that for an outlay of only about $2–3 million a year, the agreement is an inexpensive way to foster better relations. ☐

Correction

In last week's item about Czechoslovakia a line crept in to confuse the meaning of a sentence. Dr Zdenek Mlynar's dismissal was for being a member of the campaign known as Charter 77. Our apologies.

USSR

Professor Veniamin Levich was dismissed from his post at Moscow University in 1972 as a result of his application for a visa to emigrate to Israel. He had done definitive work in a number of fields including electrochemistry and physicochemical hydrodynamics. A number of Soviet scientists working in related fields were invited to attend a conference in Oxford to celebrate his 60th birthday. All declined. Extensive extracts from a letter explaining the Soviet attitude, and from a reply from Sir Derek Barton, Chairman of the Conference Committee, and Professor D. B. Spalding, Conference Organiser, appear below

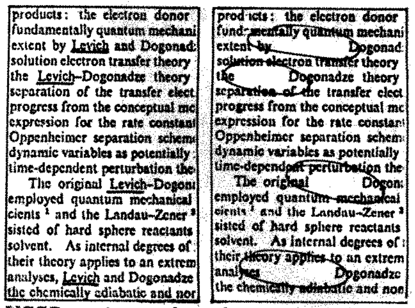
Dear Colleagues,—Over the past few months many of us have received invitations to an international conference in mid-July on "Physical Chemistry and Hydrodynamics" organised in connection with the 60th birthday of Professor V. G. Levich, corresponding-member of the USSR Academy of Sciences. For a number of reasons that are outlined below we had not intended to reply to the invitations. However, since our silence could have been misunderstood and misinterpreted, we decided to explain our attitude in this letter. . . .

You will agree that it is most unusual for a scientific conference marking the 60th birthday of a Soviet scientist, a corresponding-member of our Academy of Sciences, to have been organised and to be held in another country, without any prior consultation or agreement with organisations representing Soviet science. There is no denying that there are many major scientists in our country whose work has received universal recognition in the world. A number of them have won the Nobel Prize. But none, to our knowledge, has won such attention as Professor V. G. Levich. That leads us to believe that in this case it was not Professor Levich's scientific services that prompted this attention. Scientific services alone would not warrant it. Therefore, there must be some other reason. Apparently, it is because over the past few years Professor Levich has been busy besmirching his country, clearly to the detriment of his scientific work.

We cannot, naturally, exclude the possibility that many of the participants in the conference, and perhaps its organisers as well, honestly think there has been prejudice in Professor Levich's treatment by the leaders of his Institute, of the Academy of Sciences and perhaps also other bodies in our country. Maybe that prompted them to take such an unusual step in what they regard as support or defence of Professor Levich. But even if that were the case, it would be interesting to know their source of information.

Why, when inviting us to the conference, did nobody ask for our opinion? Why was it decided to accept implicitly the information received from Professor Levich, while the views of those who worked with him and who could provide a more objective assessment were ignored? We, consequently, feel justified in concluding that our point of view does not interest the organisers of the conference, that they are not interested in the truth of the matter, and that the entire Levich affair is merely a peg on which to hang efforts to set the scientific world in the West against the Soviet Union.

This conclusion is inescapable. It is confirmed by the wide circle of persons invited to the conference. Hundreds of people in our country, and maybe even more, have received invitations. Never before, not even when jubilees of the most outstanding Western scientists were marked, did that happen. What is significant is that many of the recipients of invitations never had anything to do with the theme of the conference, let alone the field of research in which Professor Levich works or science in general. So what does all this mean?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USSR version on the right

. . . We feel that the whole affair of marking Professor Levich's birthday can do nothing but harm to international cooperation. If the organisers of the conference sincerely desire to strengthen international cooperation, we cannot but ask—with whom? With those who, like Professor Levich, believe for one reason or another that they have been unfairly treated and have become openly hostile to our country, or with the vast majority of scientists who really represent Soviet science and are deeply loyal to our country?

We firmly believe that in deciding to convene this conference its organisers were concerned not with promoting international cooperation between scientists, but with using Professor Levich's 60th birthday to set public opinion against our country . . .

Ya. Kolotyrkin, K. Gorbunova, B. Kabanov, V. Losev, N. D. Tomashov, N. Shumilova, V. Dmitrenko, N. Lidorenko, S. Karpachoy, J. Matulis, D. V. Sokolsky, J. Stradins. Y. Delimarsky.

Dear Colleagues,—We have been gratified by the interest which you have shown in the Levich Conference. Other Soviet scientists have written in answer to the invitations; some have been friendly, and others less so; but none have set out their views at such length. Your courtesy is much appreciated. . . .

. . . We remark with pleasure that recognition of Professor Levich's contributions to science is not something which divides us.

Concerning paragraph 3 [2 above], we do agree that the holding of this conference in Oxford rather than Moscow is somewhat unusual. Had the USSR Academy of Sciences announced that it intended to celebrate the occasion suitably, the Oxford Conference might not have been convened. Were there any such intentions? Our enquiries revealed none.

We do not know how to compare the attention accorded to the Levich Conference with those celebrations which other scientists have enjoyed; but it is gratifying to the Conference secretariat to know that their publicising efforts have been successful. Our own belief is that the popularity of the conference has much to do with the high importance of physico-chemical hydrodynamics. This subject is, for many scientists, inevitably linked strongly with the work of Levich.

Your paragraph 4 questions the sources of information, and the motives, of the organisers and sponsors of the conference. Our information sources are too numerous to list comprehensively; so examples must suffice. The way in which its Soviet distributors excised Levich's name from an article by Professor Parbury Schmidt (see appendix) is documentary proof of a kind. We have seen the letter from Academician Frumkin relieving Professor Levich from his position, solely because he had exerted his legal right to apply for an exit visa. We have corresponded and held discussions with numerous Soviet scientists, officials and ordinary citizens. . . .

When, we ask you, was Professor Levich last permitted to conduct a seminar in his Institute? When will that permission next be given?

Concerning motives, it is necessary to be blunt: your "inescapable conclusion" is ludicrous; "to set the scientific world in the West against the Soviet Union" is as remote from our intentions as it is beyond our powers. We earnestly ask you to give weight to an alternative possibility, namely that the motives of the sponsors and organisers are commensurate, in probity and good sense, with their scientific attainments and with the dignity of their offices. Please consider well their names; then ask yourselves again: *can* your inescapable conclusion be correct? We would assure you that if any scientist of the standing of Levich, in any other country of the world, were treated in the same way, our involvement would be exactly the same.

In paragraph 5, you express uncertainty as to whether the effect of the conference on international cooperation in science will be good or bad; and you ask with whom cooperation is intended by us. The answer to the second question is: we hope to cooperate with *you*, and with your colleagues in the USSR; that is why we sent you invitations, and that is why the conference publicity has been kept entirely free from words which might embarrass you.

As to the effect of the conference on relations between Western and Soviet scientists, this depends greatly upon you. We have made plain our equal concern for science and for freedom of movement in all countries. We have disclaimed all provocative intent; and, incidentally, we have provided your government, if it wished to take it, with an opportunity gracefully to extricate itself from an awkward situation . . .

Yours sincerely,

Derek Barton, Brian Spalding

EEC

Research: no stop ahead?

If the words of the European Commission are anything to go by, the EEC's involvement in science and technology needs to intensify. Whether or not the Nine member countries of the Community automatically allow such a development is increasingly open to doubt, but the Commission's ideas and hopes for the next few years are now contained in a document which it approved at the end of last month and sent to the Council of Ministers and European Parliament for their consideration.

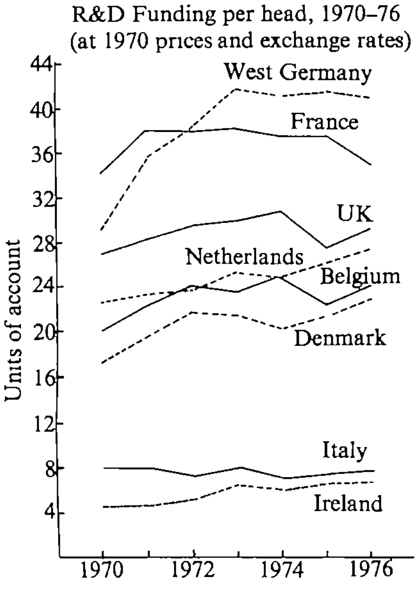
The document, *The common policy in the field of science and technology,* is in three parts. The core, appropriately enough, is the middle part, and this details the guidelines which the Commission has adopted for the next phase—that is, 1977–80—of Community science and technology policy. With ten chapters and two annexes, it is a detailed if wordy conspectus directed not only at the Council but also "to all those affected by the European research policy". According to brief final remarks, key areas of Community concern—"such as economic competitiveness, secure energy and raw material supplies and the preservation of a humane social and physical environment"—are becoming "more and more dependent" on a common science and technology policy.

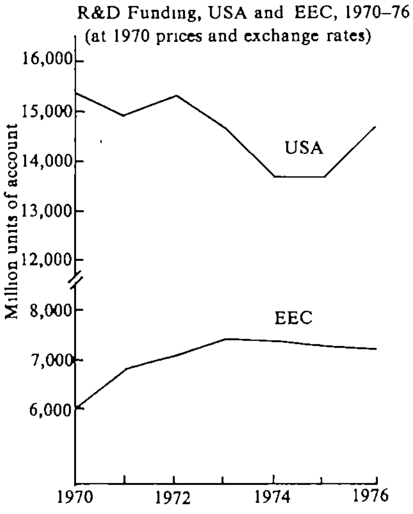
The earlier part of the document contains three draft texts which the Commission proposes that the Council should adopt. One is a resolution on the guidelines that make up the middle part This says that coordination of R&D shall be "gradually developed and intensified"; that special attention should be paid to the European Science Foundation's activities; that proposals be made for the better use of R&D results; and that a full review of EEC science activity be undertaken by the end of 1979.

The second text provides for a decision on promotion of industrial R&D projects. This would be done through financial assistance to small- and medium-sized firms cooperating in the sphere of innovation. The third text, which reflects the Commission's feelings that research in forecasting and assessment is essential, provides for a decision on a five-year research programme costing 4.4 million units of account and employing 10 staff. The aim of the programme would be "to contribute to the definition of long term R&D objectives and priorities".

The final part of the document, from which the accompanying graphs are taken, analyses public financing of R&D and has 15 graphs and four tables that summarise the main trends in the Community over the past six years. They belie the promise of the guidelines. According to the document, there is a general stagnation in R&D funding in all Community countries, an increasing gap between the Nine and the USA, and a gap between collective Community funding of R&D and funding within the individual member countries themselves. Some 64% of Community R&D funds is concentrated on energy; health takes 15–16%, and industry 12–14%.

Chris Sherwell


R&D Funding per head, 1970–76 (at 1970 prices and exchange rates)


R&D Funding, USA and EEC, 1970–76 (at 1970 prices and exchange rates)

JET

Is it enough?

A new development came last week in the saga over JET. Chris Sherwell reports

In what in the absence of confirmation otherwise looks suspiciously like an embarrassing climbdown, Britain last week withdrew one of its last remaining sanctions over the controversial decision on where to site the European Community's Joint European Torus (JET) fusion project. The Council of Foreign Ministers is due to make a final choice between Culham in Britain and Garching in Germany—unless it can come up with a third site as a compromise—at a make-or-break meeting later this month.

Britain has hitherto kept a reserve on the expenditure of about £145 million for the 1977–80 joint research programme of the Community, even though the research programme has itself long been agreed, as a bargaining chip over JET. The official reason has been that the research programme contains a good deal of fusion work, and Britain did not want to see decisions taken "piecemeal". But an announcement last week from Brussels, which was welcomed by the European Commissioner concerned, Dr Guido Brunner, disclosed that Britain had withdrawn her reserve "in the interests of the Community", but apparently in recognition that it had become more of a hindrance than an aid to progress on JET.

Whether the move will help Culham get JET is another question. Brunner spoke of "improving the climate of decision-making", but Britain's handling of matters like these in the past does not exactly augur well. At the energy ministers' meeting in March, for example, a concession over a scheme involving Euratom loans turned out to be rather costly when there was little of the expected progress on another important matter, the minimum safeguard price for oil. On that occasion too Britain's obstruction had by then blackened its reputation. The same could happen again over JET.

Moreover, the new concession may not be enough. In the view of some, a satisfactory decision on JET was possible when the research ministers met in March. The argument is that the political price Britain has to pay to win JET increased at that meeting when, as presidential chairman, she mishandled things and failed to call for a vote—a vote which, it is widely recognised, would then have gone in Garching's favour. ☐

BRITAIN

● Public fears in the developed world about nuclear power as no less real for the fact that the hazards of overeating, alcohol, cigarettes and automobiles may be greater. Among those fears the horror of radiation looms large, and if the risk posed by nuclear war is discounted, a nuclear reactor accident must be a favourite worry. The UK National Radiological Protection Board (NRPB) has reviewed the data describing the effects of radiation exposure on a population precisely in order to assess the biological consequences of the accidental release of radioactivity from a reactor. It published the findings last week*.

Information on the effects of radiation in man was derived from various studies—of individuals exposed to the atom bombs dropped on Hiroshima and Nagasaki, of Marshall Islanders contaminated with fall-out, of accident cases in the nuclear industry, and of patients treated by radiotherapy. This was supplemented with data obtained from experiments on animals. Exposure to radiation was presumed to occur in various ways: acute external exposure of the body, whether from the passing cloud or from contaminated ground; inhalation of activity from the cloud and ground and from contaminated food and water; and relocation of incorporated radionuclides within the body.

Depending on the radiation dose, an exposed population is expected to suffer both early ("acute") and late physical damage. Acute effects are minimal below a certain threshold dose. Above that dose, cell membrane damage allows fluids and electrolytes to leak out of the vascular system, and stem cells lose their reproductive capacity, meaning that normal functions can be impaired. The main late effect, cancer, has no threshold; damage to blood vessel linings, hyperthyroidism, cataracts and sterility may also occur.

The report suggests a threshold of about 200 rads (low LET), below which no deaths would occur, for irradiation of the bone marrow. For the lower large intestine a threshold of about 2,000 rads (low LET) is accepted, where the dose is received in the first seven days after exposure and the probability of death covers a one-year period. Pulmonary morbidity is not expected below a threshold dose over one year of 2,500 rads (low LET).

The report also details estimates concerning late effects. For death from leukaemia it gives a risk coefficient, in cancer deaths per 10^6 man rads (low LET), of 20. Other figures are: cancer of the lung, 20; bone, 10; liver, 10; gastrointestinal tract, 20; breast, 20; thyroid, 5; and all other cancers, 20. The total risk coefficient is 125. The estimates, the report says, are "considered adequate". As for hereditary disease, the report predicts a total of 57 serious cases per 10^6 man rads (low LET), of which 15 and 9 would appear in the first and second generations respectively.

● Britain and the USA are to collaborate on coal research. The chairmen of the UK's National Coal Board (NCB) and the USA's General Electric Company agreed last week to exchange technical information on ways of using coal more efficiently, particularly as an energy source. It is hoped that exchanges on coal gasification, liqueaction, fluidised combustion and the production of metallurgical fuel will eventually lead to jointly run research projects.

British technology is already being used in one US coal gasification project. Work on a gasification process, done at the NCB Utilisation Research Laboratories in Surrey in 1972, is to be incorporated into a recently-approved \$24-million design project which should eventually lead to a \$334-million plant for Illinois to extract gas and low sulphur oil from coal. The Canadians too are using British expertise in coal. The British partnership of the NCB and Woodall-Duckham Ltd is under contract from British Colombia Hydro and Power Company in Vancouver, Canada, to study the feasibility of designing a demonstration plant for cleanly and efficiently producing electricity and gas from Canadian coal.

Coal, of course, is the less controversial key to Britain's plans to bridge the so-called 'energy gap' in the 1990s (the more controversial one is nuclear power). But last week the chairman of British Gas, Sir Denis Rooke, injected his own element of controversy by dismissing suggestions that there will even be an energy gap at that time. The technologies were being developed, he said, to make substitute natural gas from coal or oil. The energy minister with responsibilities for gas said Britain was already looking to the time when it would have to rely on SNG.

● It is a commonly expressed view that Britain has not been making the most of its professional engineers over the past few years, and that little has been achieved in improving the engineer's lot, especially in manufacturing industry. Now the government has announced a Committee of Inquiry into the engineering profession. Sir Monty Finniston, formerly at the British Steel Corporation, will chair it.

The committee will look at how British industry could better use engineers of varying educational achievement. It will also examine the part engineering institutions could play in relation to the education and qualification of engineers at professional and technician level; the possibility of statutory registration and licencing of engineers in the UK; and the engineering profession in other countries, especially in the EEC. The committee's fifteen or so members have yet to be chosen, but they are expected to convene by the autumn.

Critics have said the inquiry might delay urgently needed changes. But the Secretary of State for Industry, Mr Varley, hopes that the inquiry will not be "unduly prolonged" (it is expected to last 18 months). And if the committee feels that some of its findings deserve urgent attention, it will probably be able to produce interim reports. One question is whether the committee will recommend the establishment of a body to control academic standards and entry to the profession. Professional engineers would then be organised in a similar way to the medical profession, and the new body would be analogous to the General Medical Council.

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* H. Smith and J. W. Stather, *Human Exposure to Radiation following the Release of Radioactivity from a Reactor Accident: a Quantitative Assessment of the Biological Consequences* (NRPB-R52, HMSO, 50p).

FRANCE

Mixed fortunes

French nuclear fortunes followed a mixed path last week. A correspondent reports

THE French Cabinet was apparently expected to discuss nuclear issues last week, but reportedly failed to do so. The minor controversy caused by a leak of uranium hexafluoride gas at a plant in southern France couldn't have helped. But there was some glory to bask in too—the signing of a cooperation agreement with West Germany covering fast breeder reactors.

The agreement is not unprecedented, being in line with an arrangement reached in Nice in February last year which involved the two countries pooling risks over both breeders and high temperature reactors. Given the new US policy, however, its timing may be awkward.

A key feature of the latest agreement is the exchange of information on fast breeder R&D over a 20-year period. This involves the French Atomic Energy Commission (CEA) on the one hand and Interatom, a subsidiary of the German company Siemens, and the Nuclear Research Centre of Karlsruhe on the other. Reports suggest that the French will maintain their fast breeder research effort at existing levels, at about FF400–500 million annually—the Germans pitch in a similar sort of amount —but that both sides are hoping for more efficient use of the funds.

Commercialisation of the fast breeder becomes the object of a new French-dominated company established under the agreement known as Serena. This allows the participation of Belgian, Dutch and Italian interests along with the principal partners from France and Germany.

Paralleling this development is one concerning French uranium supplies. In a deal with a South African corporation, the CEA is reportedly to receive 900 tonnes of uranium oxide over 10 years in return for funding the capital investment programme of the mine which will produce it. ☐

BANGLADESH

Energy projection

The Bangladesh government recently received a report on its energy prospects. A report from M. Kabir

OVERALL demand for energy is increasing with population growth and with greater industrialisation, and the government of Bangladesh has felt the need for a major study to guide long term policy and advise on specific projects that should be implemented over the next 5 to 10 years. Responding to a government request, the United Nations Development Programme (UNDP) agreed to support and finance through the Asian Development Bank a study involving Canadian, American and Italian firms. This began in mid-1973.

An 8-volume draft of the final report was submitted recently. It considers the energy sector as part of the larger economic system of Bangladesh, attempting to identify interdependence both within the energy sector and between this and other sectors of the economy.

In Bangladesh, about 90% of the people living in the villages consume about 80% of the total energy. But only a small part of total energy requirement for a massive population of 75 million is supplied in commercial form as electricity, gas or petroleum products. Most derives from the burning of traditional fuels, for which estimation of the energy equivalent is difficult given the lack of statistics.

The existing electric power system consists of two separate zones, the East Zone and the West Zone, divided by the river Jamuna. The most recent year for which data were available for the study was the financial year 1973–74, and in this period generation for public supply amounted to some 1,260 GWh—980 GWh in the East Zone and 280 GWh in the West. In addition there are facilities directly owned by users, known as 'captive power', generation of which in 1973–74 was about 460 GWh, the bulk of it in the East Zone.

In assessing demand a major effort was made in the study to project the likely growth of agriculture. That meant considering the speed with which farmers will adopt new technologies, for example, but also examining the institutional framework within which development occurs. An analysis of demand based on various 'scenarios' in a macro-economic model yielded various projections anticipating population, agricultural production and production in other sectors. The 'mid-demand' scenario, which was adopted as an appropriate basis for energy planning, projects 3,971 GWh for the East Zone and 2,292 GWh for the West. The equivalent figures on the 'high-demand' scenario were 5,004 GWh and 2,608 GWh. ☐

CHINA

Conference plan

A NATIONAL CONFERENCE on science and technology is being organised in the People's Republic of China, according to Professor Ren Ci-gong of the Applied Physics Laboratory at Johns Hopkins University. He left China on 30 June after an extended 2½-month tour. Interviewed by reporters in Hong Kong, he said its scale would "greatly exceed anything that we can imagine". During his tour he had come to know of many regional conferences, attended by specialists as well as representatives of the broad mass, and these served as preparations for the planned event.

The news accompanies intense activities in China within the past seven months both in matters of science policy and others involving modernisation of agriculture, industry, communication and defence. On the international level, a Chinese delegation which included such leading scientists as Chien San-chiang of the Peking Institute of Atomic Energy and T. C. Tung of the Institute of Zoology has just returned from a visit to Australia. And last month a team of American scientists headed by Philip Handler, President of the US National Academy of Sciences, went to China.

One of the aims of these exchange visits is no doubt to lay the path for further increases of mutual understanding and cooperation in the field of the natural sciences between the Chinese and other peoples. A long article appeared in *The People's Daily* (Peking) on 30 June signed by the 'Theoretical Group of the Academia Sinica'. In it the validity of a previously published document, "Summary of Reports from the Academia Sinica", was re-affirmed. This was frequently referred to in the campaign to criticise Teng Hsiao-ping, with whose explicit approval it was originally released for internal circulation among state departments. Its content is now widely known and may prove to be a good indication of China's science policy now that the so-called Gang of Four and their followers have been removed from the leadership.

T. B. Tang

IN BRIEF

Azbel in Israel

Mark Azbel, who for the last 2½ years has been running the Moscow 'Sunday seminar' for refusnik scientists, arrived in Israel last week. He said the recent closure of the seminar was not a condition of his obtaining an exit visa, and that he was confident it would reopen shortly.

German report

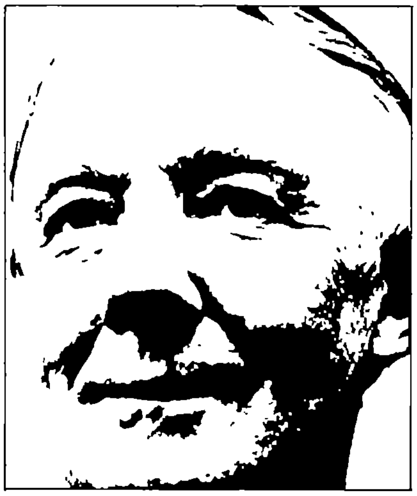
Professor Heinz Maier-Leibnitz, president of the Deutsche Forschungsgemeinschaft (DFG) has told of the continuing effect of financial cutbacks on Germany's research effort. Writing in the 1976 annual report, published at the end of last month, he says the "awareness of being at the mercy of something arbitrary in [research] promotion, wherever it comes from, and not to know any more whether a good plan will even be welcomed, easily leads to discouragement and bitterness".

Of the DM646.1 million the council had at its disposal last year (an increase of DM26.1 million on 1975), DM367.6 million came from the Federal government and DM269.9 million from the Lander.

European scrutiny

Well over a year after welcoming the suggestion that other House of Commons Select Committees apart from the existing Committee on European Legislation should consider proposals coming from the European Commission, the Select Committee on Science and Technology has decided to "consider the merits of EEC instruments falling within their order of reference". But the committee's special report announcing the decision, published last week, indicates the sort of fears about divisions of responsibility that may have caused the delay. Such work, the report insists, "cannot supplant the examination of such instruments by parliamentary representatives in the Energy and Research Committee and other appropriate Committees of the European parliament".

Acid rain



KENNETH MELLANBY

Air pollution has both local and international effects. Smoke and dust are usually urban problems, less serious the further one moves from their source. Fluoride, from brickworks and aluminium smelters, seldom causes damage beyond a radius of fifteen kilometres. But sulphur can be transported for hundreds of kilometres, over frontiers and oceans, and may cause international disagreements. It is well known that scientists in Norway and Sweden complain that sulphur emissions from Britain, Germany, Czechoslovakia and Poland have turned their rain and their rivers acid, harming freshwater fisheries, and possibly reducing growth in their coniferous forests. Many people have the impression that the industrial countries have developed a technique for keeping their own air relatively clean and at the same time causing gross pollution to harm the innocent Scandinavians.

It is true that, though there has been little decrease in the total emission of sulphur by industry and from domestic heating in Britain over the last twenty years, levels of sulphur dioxide, SO_2, in our cities have fallen as the gas has been increasingly discharged from high chimneys. This can be shown by the way the disease 'black spot' has returned to damage roses in urban gardens, where previously the fungicidal properties of the air kept it in check. But though the sulphur is certainly more widely dispersed, the bulk of it remains near to its source. British or German air is much more heavily charged with sulphur than that which crosses the sea to Norway and Sweden. British rain is generally acid. Even in rural Cambridgeshire measurements of pH 4 and lower are common; some 70 kg of sulphur is deposited on average on every hectare of Britain, and more than 20 kg in the most rural farming areas. The amounts deposited in Scandinavia are much lower. Yet damage in Britain is rare, and widespread in Norway and Sweden. The situation is clearly complicated.

In Britain most of the sulphur polluting the atmosphere is gaseous SO_2. Even in the cleanest areas annual average levels of $20\,\mu g\,m^{-3}$ are recorded. These are generally believed to have no harmful effects. None have been detected with crops or animals, and even foliose lichens, plants known for their extreme sensitivity to sulphur, flourish. In southern Norway, SO_2 levels are seldom as high as $5\,\mu g\,m^{-3}$, and, as we would expect, lichens grow well. By British standards, the air is remarkably pure. The SO_2 hardly seems to be the villain.

The main cause of acid rain seems not to be SO_2 but sulphate. In Britain amounts of sulphate are, in comparison to SO_2, low, and probably of little importance. In Scandinavia most of the airborne sulphur is present as sulphate, much derived from SO_2 produced by European industry and transformed during the period of passage north. This is washed out by the heavy rainfall. As already mentioned, the rain is actually less acid than that falling in Britain, and the total amounts of sulphur are generally small (less than 10 kg per hectare). Why then should there be all this fuss?

There are two main reasons. First, most water in Britain is well buffered, and it and most soils can neutralise most of the (comparatively large) amounts of sulphur pollution. Most Scandinavian freshwater is very 'pure' with little buffering power, so a little additional acid can greatly reduce the pH Also much rain is stored as snow for up to six months; when it thaws the pollution of half a year can be suddenly discharged.

There is no easy solution. Industrial countries can reduce levels of sulphur emission sufficiently to eliminate damaging pollution within their own borders, but will find it difficult to prevent the small fractions of their emission which escape overseas from damaging the rivers of Scandinavia. It is ironical that this sulphur can also contribute to agricultural productivity. Many Scandinavian soils are sulphur deficient and to optimise crop yields this element must be added as a fertiliser unless adequate amounts are deposited from the atmosphere. What is pollution for the fish may be an essential growth factor for the cereals.

correspondence

Preparing scientific papers

Sɪʀ,—A great deal of time is wasted because young scientists submit papers for publication in an unacceptable form. There are many good books on the market on the preparation of scientific papers, but few specific examples as to how an unacceptable manuscript can be transformed into one acceptable by leading scientific journals. By a lucky chance (and completely legally) I was able to obtain a copy of such a manuscript with the referees comments and a model re-written version, from the editorial files of a leading geological journal, and I pass this on in the hope that it will be of value to authors in preparing papers for publication.

Columnar Rock Structures from an Antique Land

Referees' report:
manuscript 19705B/76: P. B. Shelley

Manuscript as submitted:

Ozymandias[1] by P. B. Shelley[2]

I met a traveller[2] from an antique land[3]
Who said: Two[4] vast[5] and trunkless legs[6] of stone[7]
Stand in the desert.[8] Near them[9] on the sand[10]
Half sunk, a shatter'd visage lies whose frown
And wrinkled lip and sneer of cold command
Tell that its sculptor well those passions read
Which yet survive stamp'd on these lifeless things,
The hand that mock'd them and the heart that fed;[11]
And on the pedestal[12] these words appear:
"My name is Ozymandias, King of Kings.
"Look on my works, ye mightly, and despair."[13]
Nothing beside remains[14]; boundless[15] and bare
The lone and level sands[16] stretch far away.

Referees' comments

[1] This title is quite inadequate. Includes no keywords. See suggestion below.

[2] Since this paper appears to be based on field observations by another geologist, we suggest joint authorship would be appropriate.

[3] Specify.

[4] This is the only quantative statement!

[5] Not specific enough. The authors should give dimensions in SI units. (Unless "vast" is a class in some sort of grade-scale, in which case, the reference to this scale should be given.)

[6] Have alternative hypotheses been considered? Earth pillars? Basalt columns? Ant hills?

[7] Surely identification of rock type with appropriate analyses could be provided here?

[8] Give co-ordinates.

[9] Specify distance. A photograph (giving scale) would help here.

[10] Give granulometric analysis, and preferably some scanning electron microscope photographs of grain-surface textures. These don't actually prove anything, but are decorative and keep SEM operatives in employment.

[11] This fanciful and speculative section could well be omitted.

[12] This is the first we have heard of a pedestal!

[13] While it may be worth-while to record the defacement of an interesting exposure, it is not necessary to quote the words. (Since they are in English, they are obviously of no archeological interest. Presumably graffiti sprayed on by a tourist).

[14] Rather dogmatic. Better: "No other rock exposures *were observed*".

[15] Inappropriate hyperbole. The approximate extent of the desert should be stated, if relavent.

[16] Unless this is a windless desert, surely a sandy desert should show dune formation? If actually level, perhaps in fact it is a stony desert?

General remarks Although some interesting observations are recorded, we cannot recommend publication in the present form. For one thing, the paper is far too short. For another, the authors have inexplicably left out any mention of plate tectonics! For the guidance of the authors, we give below a summary of the kind of re-written and expanded paper which might be acceptable. We have had to supply necessary missing data arbitrarily; it is, of course, up to the authors to substitute the correct data.

Suggested re-written manuscript (summary)

Twin limb-like basalt columns ('trunkless legs') near Wadi Al-Fazar, and their relationship to plate tectonics

Ibn Batuta[1] and P. B. Shelley[2]

In a recent field trip to north Hadhramaut, the first author observed two stone leg-like columns 14.7 m high by 1.8 m in diameter (medium vast, ASTM grade scale for trunkless legs) rising from sandy desert 12.5 km southwest of Wadi Al-Fazar (Grid 474 753). The rock is a tholeiitic basalt (table 1); 45 analyses by neutron activation technique show that it is much the same as any other tholeiitic basalt (table 2). A large boulder 6 m southeast of the columns has been identified as of the 'shattered visage' type according to the classification of Pettijohn (1948, page 72). Granulometric analysis of the surrounding sand shows it to be a multimodal leptokurtic slightly positively skewed fine sand with a slight but persistent smell of camel dung. Four hundred and seventy two scanning electron photomicrographs were taken of sand grains and 40 are reproduced here; it is obvious from a glance that the grains have been derived from pre-cambrian anorthosite and have undergone four major glaciations, two subductions, and a prolonged dry spell. One grain shows unique lozenge-shaped impact pits and heart-like etching patterns which prove that it spent some time in upstate New York.

There is no particular reason to suppose that the columns do not mark the site of a former hotspot, mantle plume, triple junction, transform fault, or abduction zone (or perhaps all of these).

Keywords: plate tectonics, subduction, obduction, hotspots, mantle plume, triple junction, transform fault, trunkless leg, shattered visage.

[1] *School of Earth & Planetary Sciences, University of the Fertile Crescent.*

[2] *formerly of University College, Oxford.*

Yours faithfully,
N. S. Hᴀɪʟᴇ

University of Malaya,
Kuala Lumpur, Malaysia.

The laetrile question

Sɪʀ,—When Thomas H. Jukes came to witness our laetrile hearings (19 May, page 201), he missed the main battle and reported only the smokescreen. As an American, a scientist and a nonbeliever in laetrile, let me give an alternative view.

Support for freedom to use harmless drugs is no more a "crushing defeat to science and rationality" than is freedom to seek medical miracles through an established religious faith. The laetrile controversy brings into play three considerations:

- History is replete with examples where the consensus of established scientists was wrong.
- There is considerable resentment of the wealth that medical doctors extract by virtue of their monopoly control over the keys to life and health; prescriptions, laboratories and the like.
- Many people in the United States strive for freedom from excessive government; we seek a compromise between freedom and control in which individuals can pursue happiness as they see fit.

The laetrile question strikes all three chords. "Shall the government grant to doctors the exclusive right to dispense or withhold a drug which they believe to be harmless?" Would Jukes turn people away from Lourdes? Surely the answer is no, and just as surely the answer is no defeat for science.

Yours faithfully,
Tʜᴏᴍᴀs A. Cʀᴏғᴛ

Atherton, California, USA

news and views

Adenovirus amazes at Cold Spring Harbor

from J. Sambrook

The XLII Cold Spring Harbor Symposium on Quantitative Biology, entitled Chromatin, was held on 1–8 June, 1977. New insights into the ways in which gene expression is controlled in adenovirus were reported at the symposium and are described below.

IF success were exactly proportionate to effort invested, our understanding of the mechanism of gene expression in eukaryotes would be virtually complete. Unfortunately, despite intensive investigations for a number of years, our ignorance remains almost total. In part, this frustrating situation stems from the technical difficulty of analysing the expression of genes which may be represented only once in a huge and complex genome. However, the continuing dearth of useful mutations in control regions and the failure to reconstitute *in vitro* systems in which synthesis of mRNA or its precursors is initiated have also been strong contributing factors.

Despite these difficulties, there is circumstantial evidence of several sorts to indicate that extensive control of gene expression occurs at the level of mRNA synthesis. For example, mRNAs which code for specific differentiated products are detectable only in those cells which are committed to make those products. Thus, globin mRNAs are found only in reticulocytes, ovalbumin mRNAs only in cells of the oviduct and so on (see *Nature, News and Views* **267**, 203; 1977). Two competing theories have been used to explain how such gene regulation may occur. The first, based on experience with prokaryotic systems proposes that different regions of the cell's genome are transcribed into mRNA at different frequencies and that mRNA is either the direct product of the transcription event or is derived from precursors which need not be much larger than the mature mRNA itself. This scheme places control of gene expression firmly at the level of transcription, implies that there are control elements before each gene, and relegates post-transcriptional modification to a subsidiary role. The second hypothesis on the other hand suggests that mRNAs are derived from extremely long precursor molecules (identified with heterogeneous nuclear RNA) by a complex set of post-transcriptional steps involving specific endonucleolytic cleavage and modification of the 3′ and 5′ ends of the RNA by addition of poly(A) and methylated bases. This pathway, in which less emphasis is placed on transcriptional control, is attractive because it provides a plausible reason for the existence of mRNA.

To test these ideas, several groups of workers have studied gene regulation in animal viruses as a model system which might be expected to reflect the processes that occur in eukaryotic cells. Recent findings from one of these groups indicate that both mechanisms of control may be active.

Most of the data have been obtained with adenovirus 2, a human virus which grows well in cultured cells. Its genome consists of a linear complex of DNA about 35,000 base pairs in length, sufficient to code for 30–40 polypeptides of average size. The expression of these polypeptides is under temporal control: some of them (confusingly called early proteins) are synthesised at all times during lytic infection; others (late proteins) are sythesised only at late times. The early genes are collected into four widely-spaced regions and during the early stage of infection the only virus-specific mRNA present in the cell is complementary to one strand of the DNA sequences of these four regions (for a review see Flint *Cell* **10**, 153; 1977).

At the Cold Spring Harbor symposium, J. E. Darnell (Rockefeller University) presented evidence to show that each of these four groups of early genes has at least one promoter sequence. Nuclei were isolated from cells early during lytic infection and incubated in the presence of radioactive precursors of RNA in conditions which do not allow the initiation of new RNA chains, but merely the extension by a hundred or so nucleotides of those already begun in the intact cell. At the end of the brief incubation period the RNA was extracted and divided into different size classes by sedimentation through sucrose gradients. The shortest RNA molecules to be labelled *in vitro* should be those that had just begun to be synthesised *in vivo*. By hybridising the short RNA chains to different segments of the viral genome, obtained by cleavage of adenovirus 2 DNA with restriction endonucleases it was a straightforward task to identify the sites at which RNA synthesis had begun. Darnell and his coworkers (R. Evans, N. Frazer, S. Goldberg, T. Weber and N. Wilson) found four starting points for early RNA—two on the *r* strand at positions 1.8 and 80 and two on the *l* strand at positions 98 and 72. Because they lie at the beginning of the genes which code for early proteins it seems likely that these four regions serve as classical promoters—sequences which direct RNA polymerase to begin synthesis of an RNA chain. When infection is stringently blocked in the early phase, no viral RNA sequences are detectable in the nucleus other than those copied from the early regions. Thus expression of early viral genes seems quite clearly to be under transcriptional control.

At late times after infection a very different situation obtains. After viral DNA synthesis begins there is a vast increase in the rate of viral RNA synthesis as well as changes in the pattern of transcription. The cytoplasm contains a variety of new RNA species 1,000–4,000 bases long, that together account for virtually all the information of the viral genome. In addition the nucleus contains high molecular weight, virus-specific RNA molecules, some of which seem to be more than 20,000 bases long. More than 80% of both nuclear and cytoplasic transcripts are complementary to one strand of

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viral DNA—the *r* strand. In two kinds of experiment described in the April issue of *Cell,* Darnell and his coworkers (Weber, Jelinek & Darnell *Cell* **10,** 611; 1977; Goldberg, Weber & Darnell *Cell* **10,** 617; 1977) have shown that the synthesis of the vast majority of the giant nuclear viral RNA molecules is initiated at a specific site on the adenovirus genome. Once again nuclei were isolated, this time from cells in the late stages of infection, and RNA chains were extended by the addition *in vitro* of a few hundred labelled nucleotides. The radioactive label incorporated into the longest RNA molecules was found to hybridise only to the right hand end of the viral genome: the shortest molecules, however, hybridised preferentially to a region mapping between positions 14.5 and 19.0 on the viral genome. Thus late viral RNA complementary to the *r* strand is synthesised from one long transcription unit, with a promoter near position 14.5 and a terminator near the right hand end of the viral DNA. This conclusion is confirmed by experiments in which infected cells were exposed to ultraviolet irradiation —a treatment which causes lesions in DNA through which RNA polymerase cannot pass.

The frequency at which a particular sequence of DNA will be transcribed will therefore depend on whether a lesion is introduced between the sequence in question and its promoter. If the lesions are randomly distributed throughout the population of viral DNA molecules, transcription should decrease exponentially with distance from the promoter. Late adenoviral RNA synthesised immediately after irradiation was found to hybridise most efficiently with sequences from the left hand 30% of the viral genome and progressively less well with sequences to the right. The results indicate that a late promoter is present near the left hand end of the *r* strand of adenoviral DNA.

Taken together, both types of experiment show, first, that late in adenoviral infection, transcriptional control still occurs, and second, that the site at which RNA synthesis begins is quite specific and efficient. By contrast to the early promoters however, the late promoter near 14.5 on the *r* strand is highly polar and controls the expression of all genes to its right. The primary product of transcription is a long RNA molecule complementary to DNA sequences between 14.5 and 100, which is cleaved endonucleolytically to yield mature mRNA. The shift from the early to the late phase of adenovirus transcription must therefore involve alterations not only in the genes that are expressed but also in the method by which their expression is controlled.

Mass of Saturn's rings

from David W. Hughes

MASS is one of the more awkward quantities to measure in an astronomical context. Usually it is derived from a careful analysis of the perturbing effect the gravitational field of one body has on the orbit of another or, less satisfactorily, by simply multiplying the volume of the object by an estimate of its density. Inevitably the problems and uncertainties increase as the body becomes smaller or more tenuous, so W. I. McLaughlin and T. D. Talbot from the Jet Propulsion Laboratory, California are to be congratulated on obtaining the reasonably precise value of $(3.5\pm1.4)\times10^{24}$ g for the mass of the rings of Saturn. Their calculations have been published in a recent edition of the *Monthly Notices of the Royal Astronomical Society* (**179,** 619; 1977).

The gravitational potential around Saturn is a function of the planet's mass, size and principal moments of inertia and also of the mass, size and orbits of the rings and the satellites. A knowledge of the rate of precession of the satellite orbits enables this potential to be estimated with considerable accuracy. Reliable observations of the six inner satellites, Mimas, Enceladus, Tethys, Dione, Rhea and Titan going back as far as 1888 have been analysed by Kozai (*Annls Tokyo astr. Obs. Ser.* 2. **5,** 72: 1957) and Garcia (*Astr. J.* **77,** 684; 1972) to give the precession rates of the line joining the ascending and descending nodes and also the line joining the apsides.

Using these and also values for the masses and semi-major axes of the satellites, McLaughlin and Talbot have solved the gravitational potential energy function to give values for Saturn's J_2 and J_4 (coefficients in the power expansion of the potential energy function, J_2 being directly related to the moments of inertia about the spin axis and an axis in the equatorial plane) and for the mass of the rings and of the satellite, Rhea. The first solution they presented however did not use a model for the mass distribution inside Saturn and had only a limited usefulness as far as calculating J_4 and the ring mass goes, given the latter imprecisely as $(5\pm33)\times10^{24}$ g.

A much more meaningful solution was obtained by incorporating the results of Hubbard, Slattery and De Vito's high precision modelling of the interior of Saturn (see *Astrophys. J.* **199,** 504; 1975) which led to a direct functional relationship between J_2 and J_4. The inclusion of this gives a ring mass of $(3.5\pm1.4)\times10^{24}$ g, a mass for Rhea of $(2.7\pm0.5)\times10^{24}$ g and values of $(1.664\pm0.001)\times10^{-2}$ and $-(8.7\pm0.1)\times10^{-4}$ for J_2 and J_4. The mass obtained for the rings is very close to the value obtained by Franklin, Colombo and Cook (*Icarus* **15,** 80; 1971) who used a completely different calculation based on the observations of the location of the Cassini division which occurs between the A and B ring. They found a 1,400 km discrepancy between the observed position and that predicted by assuming that the division was due to particles orbiting at that distance being in resonance with the orbit of Mimas, and thus being quickly removed by gravitational perturbation. They concluded that the shift was caused by the self gravitation of ring B thus leading to an augmentation of its outer boarder. To cause the observed shift they calculated that the rings had to have a mass not less than 3.4×10^{24} g a value which agrees surprisingly well with the McLaughlin and Talbot values of $(3.5\pm1.4)\times10^{24}$ g.

So in a mass league of planetary companions the rings of Saturn come in ninth, behind Ganymede, Triton, Titan, Callisto, Moon, Io, Europa and Titania. Also if all the mass in the rings were put together into one satellite with a density say like that of Dione and Rhea (~2.4 g cm^{-3}) this satellite would have a radius of around 700 km.

David W. Hughes is a lecturer in the Department of Physics at the University of Sheffield.

Following the leader

How mRNAs are cleaved from their nuclear precursors is totally unknown, but bizarre processes must be involved. The audience at the symposium was amazed, fascinated and not a little bewildered to learn that late adenovirus mRNAs are mosaic molecules consisting of sequences complementary to several non-contiguous segments of the viral genome. In every case so far examined, late adenovirus mRNAs contain at their 5′ ends about 150 nucleotides which are transcribed not from DNA sequences immediately adjacent to the structural gene, but from sequences which map at distant locations in the viral genome.

P. A. Sharp (Massachusetts Institute of Technology) and his colleagues, S. N. Berget, A. J. Berk and T. Harrison, described experiments in which purified mRNA coding for the major coat protein, hexon, was annealed to a restriction fragment (*Hin*dIII A) of adenoviral DNA which spans the hexon gene. When the resulting hybrids were examined by electron microscopy, only

15% were found to consist of perfect duplex structures. Protruding from the vast majority was a tail of RNA which was shown by its location and length to consist of about 150 nucleotides at the 5′ (or leading) end of the mRNA. All attempts to remove the leader sequence by incubating hexon mRNA in conditions which destroy RNA–RNA hybrids were unsuccessful, and Sharp and his colleagues were forced to conclude that the leader sequences are not complementary to DNA sequences which lie immediately upstream from the hexon structural gene. The full complexity of the situation was not apparent until hexon mRNA was hybridised to the isolated *r* strand of *Eco*RI fragment A DNA (see Fig. 1). Consistently observed were hybrids whose structure could be interpreted only as shown in Fig. 2*a*.

These results mean that mature hexon mRNA consists of four separate blocks of sequences. Measurement of the lengths of single-stranded DNA and DNA–RNA hybrids show that closest to the 5′ end of hexon mRNA is a small number of nucleotides complementary to position 16.8 on the *r* strand of the viral DNA: next come approximately 80 nucleotides coded by position 19.8; then 110 nucleotides from position 26.9 and finally the coding sequences of the hexon gene itself which maps between positions 51.7 and 61.2. The structure of the hexon mRNA is shown in Fig. 2*b*.

An identical conclusion was reached independently and simultaneously by a number of groups working at Cold Spring Harbor itself, using several different late adenoviral messengers and a variety of different assay systems. Their results may be summarised as follows:

- many if not all adenoviral late mRNAs contain an identical capped oligonucleotide (7Me Gppp ACU $[C_4U_3]$ Grp) at their 5′ end (R. E. Gelinas, R. J. Roberts)
- these capped structures are not complementary to viral DNA sequences near the major late structural genes but hybridise with DNA sequences which map between positions 14.7 and 21 (D. S. Klessig)
- many late adenoviral mRNAs which function in a cell-free system, hybridise not only to the appropriate structural gene, but also to DNA sequences which map between 16 and 32 (J. Lewis, A. Dunn, J. Hassell)
- mRNAs for fibre, 100 K, hexon and penton each contain sequences at their 5′ ends which can be seen by electron microscopy to hybridise to positions 16.7, 19.7 and 26.7 on the *r* strand of the adenoviral genome (L. T. Chow, Gelinas, T. R. Broker & Roberts).

These unexpected findings raise a number of interesting questions. How are the mosaic molecules synthesised? Why are the three leader sequences separated from each other and from the structural genes? Do the leader sequences serve any function? How general is the phenomenon?

At the symposium four possible ways were discussed by which mosaic late adenoviral mRNA might be synthesised: first, the polymerase responsible for late adenoviral transcription might jump from one segment of the viral genome to another in an ordered fashion. Second, there might be new DNA templates generated, perhaps by recombination, that would contain the appropriate arrangements of different segments of the viral genome. Third, each of the segments may be transcribed independently and the mature mRNAs may be synthesised by ligation of components. Fourth, all late mRNAs that contain an identical leader sequence may be derived by cleavage from the same nuclear precursor.

The first three hypotheses are inconsistent with the data of Darnell's group discussed above. The fourth hypothesis, however, is identical to the conclusions drawn by Darnell and his coworkers. All the mRNAs that have been shown so far to share the same leader sequence also belong to the same transcription unit and fall under the control of the promoter located on the *r* strand of viral DNA near position 14.5. By contrast a small late mRNA coded by sequences mapping between positions 9.7 and 11.1 on adenovirus 2 DNA and whose synthesis cannot be controlled by the promoter at position 14.5, contains a leader sequence which hybridises to DNA between positions 5.0 and 6.4 (Chow, Broker, Roberts & Westphal).

The best mechanism to explain these findings seems to be that espoused by Klessig. He believes that there may occur in the primary transcript, base-

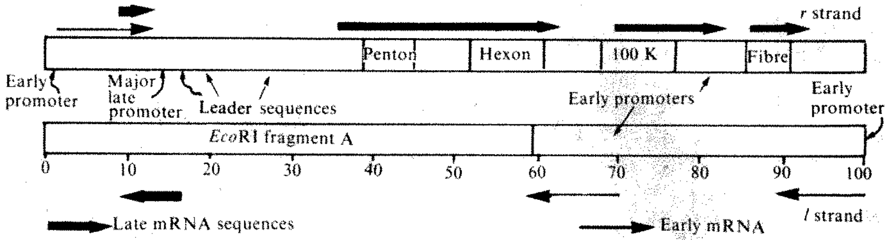

Fig. 1 Transcription map of adenovirus 2 (see Flint *Cell* **10,** 153; 1977).

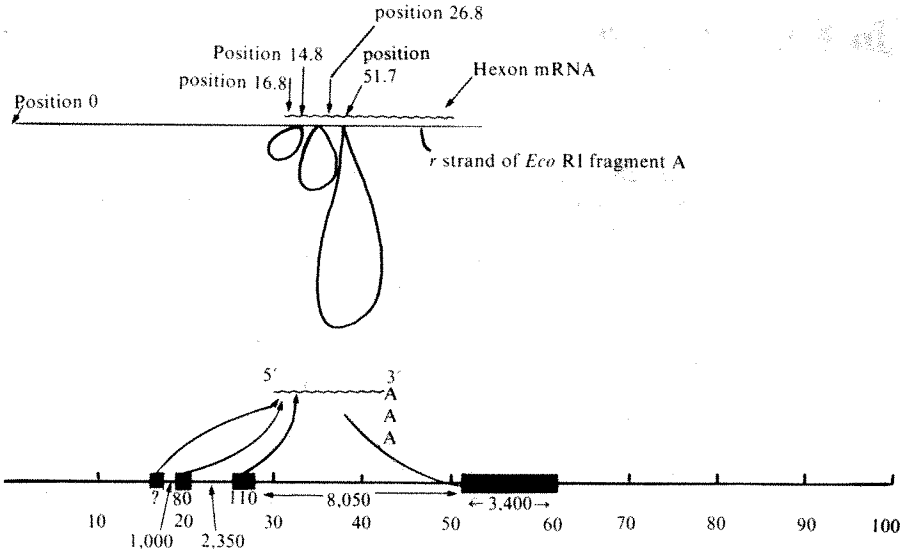

Fig. 2 *a*, Pattern of hybridisation between hexon mRNA and the *r* strand of *Eco*RI fragment A of adenovirus 2 DNA. *b*, Regions of adenovirus genome which contribute to hexon mRNA. Figures other than adenovirus DNA markers represent distances in nucleotide base pairs.

pairing between the leader sequences and the coding sequences so that mature mRNA would be derived by recombination-like events. Each precursor would then generate one mature mRNA. Perhaps separation of the several leader sequences and those of the structural genes allows several different modes of base pairing whose relative frequencies may determine the rates at which different mRNAs are produced.

The major problem is to find out how widespread the phenomenon is. Already there is evidence presented at Cold Spring Harbor by S. Weissman, Y. Aloni, G. Khoury and Ming Ta-Hsu that a similar situation may occur during synthesis of late SV40 mRNAs. But the key question is whether analogous events happen in uninfected cells. Perhaps it is not too optimistic to hope that what has been so clearly shown for adenovirus 2 may also help to explain how host cells regulate their own genes. □

Genes, channels and membrane currents in *Paramecium*

from Roger Eckert

THE ciliated slipper animalcule first observed by Anton van Leeuwenhoek in 1674, and familiar to generations of introductory biology classes and students of cytoplasmic inheritance, has lately proved increasingly useful for novel interdisciplinary approaches to problems of broad neurobiological interest. This is not a completely new departure, however, for the very first intracellular electrical measurements from animal cells were made on *Paramecium* over 43 years ago by T. Kamada (*J. exp. Biol.* **11**, 94; 1934), a pioneer in the use of saline-filled capillary microelectrodes. More recently the surface membrane of this unicellular organism was found to exhibit properties of electrical excitability (see review in *Calcium in Biological Systems* ed. Duncan, C. J. page 233 Cambridge University Press, 1976), similar in essence to those shown by metazoan nerve and muscle preparations. Most prominent is a graded depolarisation produced by an inward regenerative current in response to depolarising stimuli. The entry of Ca ions carrying this current produces a transient rise in intracellular [Ca] that mediates a reorganisation of axonemal activity seen as reversed ciliary beating with backward swimming. The stimulus–response coupling sequence is summarised in Fig. 1.

As in the classical excitable membranes of nerve and muscle, depolarisation leads to an inward current (carried in *Paramecium* by Ca) followed by a more slowly developing outward K current. The electrically excitable membrane channels that carry the Ca current seem to reside almost exclusively in the portions of the surface membrane covering the cilia, for removal of the cilia eliminates the inward Ca current, which is restored gradually as this portion of the membrane regenerates with ciliary regrowth over a period of several hours (Dunlap & Eckert *J. Cell Biol.* **70**, 245a; 1977). Interest in Ca channels is heightened by reports of their occurrence and functioning in sensory, nervous, secretory, embryonic and motor tissues, and by growing evidence that Ca entering cells often acts as a second messenger in modulating certain metabolic activities as well as membrane conductances (Baker in *Calcium Movement in Excitable Cells*, Pergamon Press, Oxford, page 7; 1975; also various articles in *Calcium in Biological Systems op. cit.* Cambridge University Press, 1976).

Paramecium offers a unique combination of features useful for new approaches to the study of the Ca system and other components of excitable membranes. As a microorganism it can be mass cultured, cloned and manipulated and analysed genetically. It also possesses the fundamental electrical properties of an excitable cell. Because Ca channels are localised in the membrane covering the cilia a greatly enriched channel fraction can be produced by collecting the cilia which have a high surface area/volume ratio. Regrowth of the cilia provides a model system for studies of channel regeneration and insertion into the membrane. In addition, the coupling between membrane function and ciliary activity facilitates identification and isolation of membrane mutants by their sensory and locomotor behavioural phenotypes. The sensitivity of the ciliary apparatus to intraciliary [Ca] also provides an independent assay of Ca entry through the channels of the ciliary membrane (see below).

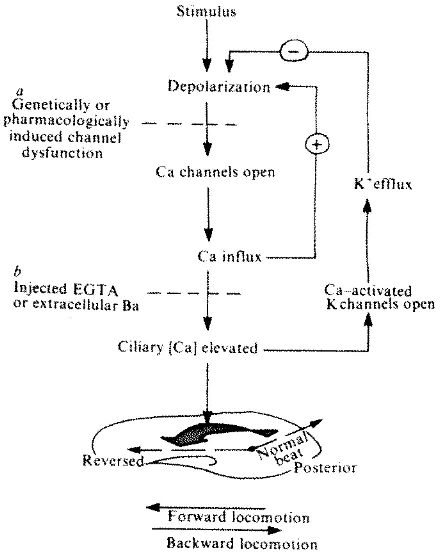

Fig. 1 Relations between channels, currents, and locomotor activity in *Paramecium*. The simulus-response coupling sequence, which proceeds from top to bottom, can be interrupted (dashed lines) in various ways; for example, by blocking the Ca channels (*a*) or by interference with intraciliary accumulation of free Ca^{2+} (*b*). Barium ions, like Ca^{2+}, promote ciliary reversal, so they do not prevent reversed swimming, although their substitution for intraciliary Ca does interfere with activation of the Ca-dependent K channels, causing Ba to promote long-lasting all-or-none action potentials with accompanying ciliary reversal in place of the brief, graded Ca response normally produced by the membrane. EGTA injection, by limiting Ca concentration buildup, interferes with both ciliary reversal and activation of the Ca-dependent K channels. This can produce prolonged all-or-none action potentials without ciliary reversal. A report in this issue (Oertel *et al. op. cit.*) demonstrates the utility of a membrane mutant in which a genetically determined Ca channel dysfunction (*a*) is used to analyse the normal behaviour of the membrane.

This issue of *Nature* contains an excellent example of the clever genetically-assisted analyses of membrane function that are being carried out on *Paramecium tetraurelia* by Ching Kung and his associates at the University of Wisconsin (Oertel *et al.* page 120). Membrane currents in response to step depolarisations of the wild type were compared with those of *pawn*, a class of mutant in which the Ca channels had been shown previously to be functionally defective. Predictably, the *pawn* membrane produced only the delayed outward K current, whereas the wild type produced, in addition, the previously reported early inward current carried through Ca channels. Averaged *pawn* current trajectories

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were graphically subtracted from averaged wild-type trajectories in order to produce a difference trajectory representing the currents that are missing in the mutant. This approach is similar in concept to isolation of partial currents with the aid of ionic or pharmacological blocking agents (Fig. 1, point *a*), which, regrettably, have not worked well to date on *Paramecium*. From these analytical procedures, Oertel *et al.* concluded that the Ca current completely and rapidly inactivates within milliseconds rather than persisting under prolonged depolarisation, and that a prolonged Ca-activated K current, which might conceal the Ca current, is also absent in the wild-type membrane.

This work is noteworthy not only because it introduces a unique and interesting genetically-assisted approach to membrane current isolation, but also because it has led to important conclusions that seem to disagree with conclusions arrived at through less elegant but perhaps equally reliable means (Fig. 1, point *b*). Thus in simple membrane voltage recordings, injection into *Paramecium* of the Ca-chelator EGTA, or extracellular addition of barium ions converts the normally brief, graded Ca response to an all-or-none action potential exhibiting prolonged plateaux due to delayed repolarisation. In molluscan neurones such behavior is ascribed to diminished activation of a Ca-activiated outward K current (Meech *Comp. Biochem. physiol.* **48A**, 387; **48A**, 397; 1974), and concomitant exposure of a persistent inward Ca current that normally is short-circuited by and can be obscured by the Ca-activated K current (Eckert & Lux *J. Physiol.* **254**, 129; 1976).

The cilia, which are sensitive to the intraciliary Ca concentration, beat steadily in reverse throughout voltage clamp depolarisations for periods lasting from tens to tens of thousands of milliseconds, but revert quickly to nonreversed beating characteristic of the low resting intraciliary [Ca] on repolarisation (Machemer & Eckert *J. comp. Physiol.* **104**, 247; 1975). This precise temporal coupling of Ca-dependent, reversed beating to membrane depolarisation in *Paramecium* suggests that a Ca current continues to flow during depolarisation, maintaining an elevated intraciliary Ca concentration. Such a Ca current can easily be overlooked in current measurements if short circuited by a Ca-activated K current (see for example Clusin & Bennett, *J. gen. Physiol* **69**, 145; 1977). Rapid shut down of Ca channels (Eckert *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 1748; 1977) could make it difficult to measure the end-of-pulse tails of a persistent current. Other factors that might contribute to apparent or real Ca inactivation are: (1) the negative-going shift in the Ca equilibrium potential with attending drop in EMF driving the Ca current that occurs as the Ca activity within the restricted space of the cilia rises during Ca entry; and (2) a possible depression of the conductance of the Ca channel due to accumulation of Ca within the channel or at sites on the cytoplasmic side of the membrane. Such self-limiting behaviour may be absent with injected EGTA or with extracellular barium.

These and similar problems in sorting out partial currents are not limited to *Paramecium*, of course, so it will be of general interest to see how they are resolved. In any event, the report by Oertel *et al.* reminds us again that genetically assisted approaches to neurobiology, first applied to metazoan material by Benzer, Pak, Nirenberg, Nelson, Brenner and their associates, are not restricted to multicellular animals that possess a nervous system. It seems likely, in fact, that combined genetic, electrophysiological and biochemical approaches facilitated by the unique and fortuitous combination of properties exhibited by *Paramecium* and its relatives will contribute significantly to our understanding of excitable membranes. □

NMR of macromolecules

from Paul Calvert

The Euchem Conference on The Study of Macromolecules by NMR was held at Grasmere on 17-21 May, 1977.

NMR is no longer of wide interest as an effect in its own right but only in so far as it can yield useful chemical information. While the Euchem Conference provided no fireworks it did give a good general view of what NMR can now say about macromolecules and what can be expected in the near future. The usefulness of NMR to the study of macromolecules has increased in recent years with the development of commercial pulsed Fourier transform instruments and high field superconducting magnet spectrometers. High field (> 6.3 T) spectrometers are required to produce well-resolved spectra of high molecular weight polymers because their relatively slow motion in solution allows proton–proton interactions to broaden the lines. (1 Tesla = 10^4 Gauss. The larmor frequency of protons is 100 MHz at 2.4 T.)

Synthetic polymers

The meeting was divided into synthetic polymers, molecular motion, and biological polymers, but in essence the subject has two branches: first, the effects of chemical modification on peaks obtained from resolved spectra, and second, determination of the side group and main chain motions from the peak relaxation times. H. J. Cantow (University of Freiburg) introduced synthetic polymer structure studies, pointing out that there were now more than 100 papers describing primary structure determination in polymers by ^{13}C NMR. ^{13}C chemical shifts of aliphatic hydrocarbons cover a wide range and are additive, which has made this the standard technique for structural characterisation of vinyl polymers and copolymers; especially in industrial laboratories for the study of the effects of polymerisation conditions on structure. For instance, in ethylene–propylene copolymers it is possible to determine the lengths of $-(CH_2)-$ runs based on additivity rules and comparisons with model compounds. The chemical shifts contain information on primary structure, configuration and conformation. Thus in a polymer such as polypropylene it is possible with ^{13}C NMR to get detailed tacticity information if conformational effects can be ignored. The snag is that configurational effects only arise because different configurations adopt different conformations in solution. Hence different solvents can give very different shift patterns and small compound model data can be misleading. The structure of the poly(diene) rubbers is more complicated with 1,4 and 1,2, *cis* and *trans*, and head–head, head–tail and tail–tail structures, so that the peak assignments are still not sorted out. Papers by K. J. Ivin (University of Belfast) on polycyclopentenes and polynorbornenes, and by F. Schue (Montpellier) and Y. Shabab (University of Surrey) on polypentadienes dealt with these aspects. Conformational effects are of great interest from the point of view of the solution and melt properties of polymers, but are in a primitive state. Cantow described attempts of his group to relate the observed temperature dependence of chemical shift to conformational energy diagrams.

While it is very nice to have detailed tacticity information, it would be good to see it put to use to further our understanding of polymerisation or polymer properties. A. Zambelli (University of Milan) showed how this can be done in experiments where, by looking at 'mistakes' in isotactic and syndiotactic polypropylenes, he was able to show that isotactic polymerisation is directed by the

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catalyst whereas in syndiotactic polymerisation it is the polymer chain which governs the orientation of a newly added unit. On occasions the results presented at the conference appeared to be a sophisticated catalogue, whereas experiments such as this present more meaty concepts.

Two papers dealt with thermosetting resins, the cinderellas of polymer science, J. R. Ebdon (University of Lancaster) on ureaformaldehyde resins and H. Pyszora (BP, Sunbury-on-Thames) on phenol–formaldehyde resins. Here NMR can only yield information about the early stages of reaction as cross linking causes loss of resolution as the molecular mobility decreases. The main lesson is that ^{13}C NMR is very useful if, as in many vinyl polymers, the tedious process of assigning peaks has already been done.

Biological polymers

Much effort in synthetic polymers is given to picking out rare events such as branches and chain ends. In proteins every group is a rare event, and the problem is first to get a resolved spectrum at all and then to get enough information to tell which peak is which. A. Allerhand (Bloomington University), Indiana showed how the non-protonated carbons of aromatic amino acids in proteins could be identified by ^{13}C NMR. These have the advantage of giving sharp peaks since they are not relaxed by attached protons, and they are spread over a wide range of chemical shift. Peaks are assigned by *p*H titration and chemical modification including use of bound lanthanide shift reagents. He described studies of tyrosines in myoglobins and histidines and tyrosines in lysozyme. The peaks get broader for larger proteins and high field instruments (> 4 T) will not improve resolution for the non-protonated carbons as chemical shift anisotropy dominates the relaxation. K. Wuthrich (STH, Zurich) showed how high field (8.4 T) proton NMR can be used to get well-resolved spectra of proteins of up to 20,000 daltons with digital resolution enhancement methods. Peaks were identified by titration, double resonance and by two dimensional Fourier transformation against chemical shift and J-coupling, to allow multiplets to be picked out. I. D. Campbell (University of Oxford) described similar experiments devised in Oxford. He showed how presaturation pulses can be used to invert and so identify multiplets and to measure exchange rates for labile protons.

These methods are most informative for proteins whose primary and X-ray structures are already known, so that amino acids can be definitely identified. Under these circumstances it is possible to study the effects of *p*H and ligands on structure and pick out hydrogen-bonded groups by deuterium exchange. In denatured proteins chemical shifts for identical residues become equal so the chemical shifts in the spectrum of a native protein must contain a great deal of conformational information which cannot be interpreted as yet. A number of papers described the interaction of ligands with proteins. J. Feeney (National Institute for Medical Research, London) discussed the binding of antifolate drugs and drug fragments to dihydrofolate reductase. The effect on histidines could be observed by *p*H titration, and on tyrosines and tryptophans by ^{19}F and ^{1}H NMR of enzymes produced by bacteria fed with fluorinated and deuterated amino acids. Binding of the NADPH cofactor could be followed by ^{31}P NMR, showing a conformational change in the pyrophosphate group and that the 2′-phosphate group binds as a dianion. In a similar vein B. Lindman (Lund University) described studies of halide binding to proteins using chlorine, bromine, and iodine NMR (see Lindman & Forsen in *Nmr Basic Principles and Progress* **12,** Springer Verlag, Berlin): it was possible to determine the stoichiometry of binding and, in the case of metalloenzymes, whether or not the halide binds to the metal. W. E. Hull, (Bruker, West Germany) described ^{19}F NMR of alkaline phosphatase to follow metal ion binding and ^{31}P to follow phosphate interactions. Weill described studies of cobalt and manganese in binding to polyelectrolytes by following the relaxation behaviour of the water. The overall message here seems to be that binding studies can be very informative if the NMR technique and ligand are well matched, and several independent measurements can be made. Otherwise there is often a large element of uncertainty as to interpretation.

Molecular motions

Since the advent of pulse Fourier transform spectrometers T_1 relaxation time measurements have become simple to carry out, and molecular motion studies have become an integral part of most macromolecule experiments. F. Heatley (University of Manchester) described studies of synthetic polymers in solution by ^{13}C and high field proton NMR. Measurements of correlation times from T_1 values and nuclear Overhauser enhancements disagree with one another to the extent that a correlation time distribution rather than a single time must be adopted. From proton relaxation times it is possible to show that a χ^2 correlation time distribution cannot fit the data over a wide temperature range, but that a molecular jump model can be used in combination with overall molecular rotation. These models seem to have great potential and are based on an enumeration of the possible conformational changes of chain segments with fixed ends. For a diamond lattice, for instance, these jumps involve motions of three and four bonds simultaneously (see Valeur *et al. J. Polymer. Sci.* (*Physics*) **13,** 667, 675; 1975).

N. Gronski (Freiburg University) and H. C. Broecher (University of Hamburg) used such models for polydienes and polyisobutylene, and F. Laupretre (Paris) for polystyrene. One criticism of this work is that there is little effort to compare the results with those from dielectric and other relaxation methods. On the biopolymer front T_1 values are extensively used to measure exchange processes and to look at phenyl group rotations, though in this latter case, it is hard to distinguish between full rotation and oscillations (see *News and Views* **258,** 112; 1975). This is an area where we can expect a lot of progress and consequently an increase in our knowledge of conformational flexibility of proteins in solution. High resolution NMR of solid polymers using a combination of high power proton decoupling and magic angle spinning was described by J. Schaeffer (Monsanto, St Louis) who related the correlation time data to the mechanical properties of the polymers (see *News and Views* (1976) **261,** 457; 1976 and *Macromolecules* **10,** 384; 1977).

NMR of polymers has often been accused of never generating indisputable information. This is becoming less true, particularly if the applications are carefully chosen. The advantage of NMR over other techniques is that resonances from individual groups are observed and can be monitored to give detailed information about their states (motional, configurational, conformational, ionisation). A disadvantage is the limited frequency range available which often can make the results ambiguous. For enzyme studies a further disadvantage is that even for ^{1}H NMR relatively large amounts of sample (0.5 ml of 0.5 mM solution) are required. ☐

Neoplastic transformation

from N. A. Mitchison

The Dahlem Conference on Neoplastic Transformation was held in Berlin on 9–13 May, 1977, and was organised by Dr Silke Bernhard. The proceedings will be obtainable from Dahlem Konferenzen, Delbrückstrasse 4c, D-1000 Berlin 33.

THE conference aimed at assessing current knowledge and tried to pose questions that would draw attention to gaps in our understanding of the process by which normal cells become tumours. One was left with the vivid impression that cancer research continues to provide an area of excitement, in which much of what is new in biology comes up for scrutiny An account of the proceedings will be published within six month. The participants mentioned in this report are not necessarily, of course, the authors of the views which they discuss.

Epidemiology was much discussed. Interest is at present greatest in environmental causes of cancer, having moved from previous concern with radiation and viruses. The strongest view (Muir) is that as much as 80% of cancer may be caused by environmental agents. The evidence comes partly from direct study of the effects of tobacco and alcohol, which are known to cause about 40% of cancers in Western males, and partly from study of particular industrial hazards, known to cause at least 1%. Comparisons between different environments tend to support the environmentalist view; the difference in cancer incidence between Mormons and their fellow US citizens, for example, is especially striking (C. S. Muir, International Agency for Research on Cancer, Lyons: R. W. Miller, National Cancer Institute, Berthesda). On the other hand it can be argued that all cancers are 100% genetic, just as they are also 100% environmental in origin. And although early pregnancy protects statistically against cancer of the breast, J. Cairns (Imperial Cancer Research Fund, London) pointed out that one cannot usefully describe the avoiding of pregnancy as an environmental cause of cancer.

Widespread use of the Ames test has confirmed the mutational origin of cancer (A. G. Knudson, Institute for Cancer Research, Philadelphia). The monoclonal nature of most tumours supports this view (P. J. Fialkow, Veterans Administration Hospital, Seattle); the exceptions to this rule are understandable, for example, hereditary multiple neurofibroma or infective warts. So also does the predisposition to cancer in inherited defects in DNA repair, particularly xeroderma pigmentosum and ataxia telangectasis. Somatic genetics has now reached the point where the first of these diseases can be subdivided into six complementation groups, and the second into three. Yet further support for the mutation view comes from the biochemistry of DNA repair: the failure to excise O^6-ethylguanine explains why ethyl nitrosourea causes tumours in the rat brain (K. Rajewsky, Universität Köln). We are left with two main questions: how important are endogenous carcinogens; those detected by mutational screening of bile or urine (P. N. Magee, Temple University, Philadelphia) for example, and do environmental carcinogens act as promoters rather than as initiators (Cairns)?

How do viruses transform cells (W. Doerfler, Universität Köln; E. L. Winnaker, Universitat Munchen; C. M. Croce, Wistar Institute, Philadelphia)? The nearest we can come to answering this question at present is to find out whether SV40 or the RNA leukaemia viruses integrate into a definite site in the host genome, presumably adjacent to the oncogene(s). The evidence is still inconclusive. Cutting the genome of SV40-transformed cells with restriction enzymes suggests that integration is promiscuous; but cell hybridisation studies indicate that integration occurs preferentially in human chromosomes 7 or 17 (Croce). Further analysis using restriction enzymes should answer the question.

A respectable case can be made for the proposition that cancer results not from a stable genetic change, but from a reversible form of differentiation. Thus, in chimaeric mice, genetic markers from teratomas have been detected in normal, differentiated tissue (R. L. Gardner, University of Oxford); however, the most dramatic finding, by B. Minz, that teratoma-derived gametes can give rise to normal offspring, has yet to be confirmed. Since at least nine different chemical factors control the growth of cells in vitro (C. H. Sato, University of California, San Diego)—and the list is rapidly growing—there is plenty of scope for control of differentiation. However, the decisive experiments have proved curiously elusive: nuclear transplantation, or the isolation of normal cells from tumours other than teratomas.

Cancer immunology survives, in spite of the collapse of immunological surveillance as a general theory. Indeed, surveillance probably does operate, but only in restricted circumstances involving viral antigens such as nasopharyngeal carcinoma of the Chinese. Up to date cellular immunology links up with cancer when the question is posed whether manipulation of the immune system may reveal otherwise undetectable responses to weak tumour antigens (B. A. Askonas, National Institute for Medical Research, London; Rajewsky; K. P. Eichmann, Institute fur Immunologie und Genetik, Heidelberg). The idiotype network, and T helper cells, provide logical handles for such manipulation. The most exciting development in cancer immunology has been the discovery that tumour specific antigens and major histocompatibility antigens interact in several ways (E. S. Lennox, MRC Laboratory of Molecular Biology, Cambridge). Thus new H-2 specificities occur on cancer cells, as a result either of derepression or post-translational modifications. This implies that the molecular biology of the tumour specific antigens may be more accessible than had been expected. ☐

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Snake's eye view of Adam and Eve

from K. W. Jones

A MOLECULAR difference at the DNA level between human males and females (surely fuel for the current debate on whether or not man is man or merely person) is the substance of recent work by Kunkel *et al.* (*Science* **191**, 1189; 1976). Males possess a Y chromosome which is absent from normal females and carries the gene(s) which determines primary sex. By reassociation of traces of male DNA with an excess of female DNA these workers have isolated a class of repeated DNA (It-Y DNA) which does not reassociate with female DNA, but which will reassociate with male DNA. It is therefore presumably of Y chromosome origin. More recently (*Proc. natn. Acad. Sci. U.S.A.* **74**, 1245; 1977) the same workers have analysed the content of It-Y DNA in the DNA from individuals with various structural abnormalities of the Y chromosome and shown that It-Y DNA comprises 15–30 different families of reiterated sequences, is largely located on the fluorescent part of the long arm of the Y and amounts to about 10% of the total chromosome. This constitutes the first characterisation of repeated DNA unique to a single human chromosome. The fact that it is on the Y chromosome immediately poses the question of its role in sex determination but, as the authors point out, such a role is rather unlikely since in their study some normal males with small non-fluorescent Y chromosomes lacked It-Y DNA whereas some females with Y translocations did possess it.

Recently Cooke (*Nature* **262**, 182; 1976) has described repeated DNA specific to human males and amounting to 50% of the Y chromosome. This was detected by digesting DNA from males and females with the restriction enzyme *Hae*III which cuts DNA at a short defined base sequence to yield fragments of different lengths defined in distribution according to the spacing of the target sequence cleaved by the enzyme. When the fragments were separated by gel electrophoresis in agarose, DNA bands were seen in the male digest which were absent from the female digest, suggesting the unique presence of a repeated sequence containing the *Hae*III target site in male and therefore in Y chromosome DNA. Since this large fraction of Y chromosome DNA is not included in the It-Y DNA recovered by Kunkel *et al.*, it must now be supposed that it is sufficiently homologous with DNA present in the female to be removed by reassociation analysis. From my own observations, Cooke's DNA fractions are located essentially in the fluorescent region of the Y along with the It-Y DNA.

Highly repeated simple sequence (satellite) DNAs in man are also concentrated in the fluorescent long arm of the Y as well as being widely represented in many other human chromosomes. The relationship of the satellite DNAs to the other Y-repeated DNAs is not known at present and their quantitative contribution to the Y DNA as a whole is also not determined. It seems however that most of the Y chromosome long arm is composed of repeated DNA and that of this, only It-Y DNA is unique to this chromosome. The implication of the finding that It-Y DNA maps to the fluorescent long arm of the Y is that the entire repeated DNA of the remainder of the chromosome, including that in the region which contains the sex-determining gene(s) is sufficiently homologous with DNA present elsewhere in the genome to be included in the fraction which reassociates with female DNA in the conditions of Kunkel *et al.*'s experiments. This is perhaps not too surprising since the X and Y chromosomes were presumably homologous at some early period in their evolution, and it has been suggested that they are derived by a process of translocation from structural homologues (see Lyon *Nature* **250,** 651; 1976).

The significance of the high concentration of repeated DNA on the Y chromosome or indeed of the significance of most non-transcribed repeated DNA is unknown. According to Miklos and Nankivell (*Chromosoma* **56,** 143; 1976) satellite DNA may play a part in controlling the frequency of crossing over between homologous chromosomes. By crossing over, pairs of chromosomes remain largely homologous through the frequent interchange of DNA. Conversely, chromosomes which fail to cross over will tend to evolve divergently. Some change inhibiting crossing over would therefore seem to be a primary requirement for the evolution of specialised sex chromosomes.

Snake's eye view

According to Singh *et al.* (*Chromosoma* **59,** 43; 1976), in the snakes, which exhibit the entire process of evolution of sex chromosomes, the first discernable change is in the repeated DNA structure of one of a pair of chromosomes bearing the sex determining gene(s). Within the snakes there is a range of forms from primitives with no defined sex chromosomes through intermediate forms with definite, though morphologically identical, sex chromosomes, (called ZW in the female, which is heterogametic, and ZZ in the male which is homogametic) to forms with highly differentiated, morphologically dissimilar Z and W chromosomes. The primitive snakes show no difference between male and female in highly repeated (satellite) DNA whereas the intermediate forms show very obvious differences involving the appearance of novel satellite DNA in the female. This novel DNA maps exclusively, by *in situ* hybridisation, to the W chromosome (which is the functional equivalent of the Y in mammals) where it is spread over the entire length. The identical-looking Z chromosome lacks this DNA. The W chromosome also shows a different cycle of DNA synthesis from the Z, as is frequently the case in other satellite-DNA-rich chromosomal regions. Singh *et al.* suggest that this interferes with pairing between the Z and W thus predisposing to their divergent evolution and accounting for the fact that in the most evolutionarily advanced group of snakes the W (like the Y in man) has become greatly reduced in size and modified in form relative to the Z. The modified W chromosome nevertheless has conserved the satellite DNA sequences found in the W of the intermediate group, as determined by molecular hybridisation, as well as its allocyclic DNA synthesis pattern. From this it may be suggested that in the human Y chromosome the plethora of simple sequence DNAs reflects the molecular end consequences of the mechanism which played a part in its specialisation. Once a chromosome, like the Y, is genetically isolated, the constraints on acquiring, or amplifying, additional non-transcribed (functionless) DNA sequences are probably lost. The Y chromosome is therefore likely to be loaded with "junk DNA" (Ohno *Hoechst Symposium;* 1972) which no longer serves a useful purpose. At a practical level, however, it now seems a feasible and exciting prospect to use the It-Y DNA as a handle to fish out covalent Y chromosome DNA sequences by some type of DNA affinity chromatography. This will shed further light on the structure and evolution of the human Y chromosome and it may ultimately permit a direct approach to the molecular basis of sex determination at the DNA level. ☐

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A hundred years ago

In the debate on the education estimates, on Tuesday night, Sir John Lubbock, speaking on the extra subjects which had been made compulsory, said he doubted whether under any circumstances it would be desirable thus to stereotype one form of education for the whole of England; but surely we ought not to do so unless we were very clear as to what is the best system. There was, however, very great difference of opinion on this head. The first authority to which he would refer was that of a committee of that House. It was presided over by his hon. friend the member for Banbury, and after careful enquiry they reported that in their opinion "elementary instruction in the phenomena of nature should be given in elementary schools." The next authority which he would quote was the Royal Commission, presided over by the Duke of Devonshire, which unanimously recommended that more substantial encouragement should be given to the teaching of the rudiments of science in our elementary schools. In Scotland, too, great dissatisfaction was felt with the present system. At the last conference of elementary teachers, held in London, which was very numerously attended, it was resolved that the system of payment "embodied in the Code is unsound in principle and injurious to the progress of true education." The inspectors of schools differed greatly as to the most suitable subjects. Even in regard to geography they were not unanimous. It was said as a subject to lend itself very much to "cram." One of the inspectors gave in support some very amusing answers. For instance, in answer to a question of "What are mountains and rivers?" one girl replied "Mountains in some parts of the world are very useful. In Africa, for instance, they shoot out gold." Of rivers she had not so favourable an opinion, though she thought "they were all very well in some countries where there was very little rain." He confessed, however, that he thought geography a very good subject, though he was not convinced that it ought to be continued during the whole course to the exclusion of other subjects. The mere skeleton of history taught in our elementary schools contained little more than dates, wars, and murders; but dates and crimes no more constituted the history of a nation than sinews and bones made a man. Men of science must be grateful to Sir John Lubbock for so constantly urging upon Government the importance of scientific education.

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review article

Transposable genetic elements as agents of gene instability and chromosomal rearrangements

Patricia Nevers & Heinz Saedler*

Transposable genetic elements in prokaryotes and eukaryotes, when inserted at a given locus, can control expression of the locus and cause large scale rearrangements of adjacent DNA sequences. Striking similarities in genetic behaviour between the two groups of elements have led to the proposal of a molecular model of eukaryotic controlling elements, and to suggestions about the part such elements may play in evolution and differentiation.

CHROMOSOMES are commonly regarded as conservative structures in which an exact amount of genetic information is arranged in a definite sequential order. This order is normally preserved when information is exchanged between chromosomes, and guaranteed by a set of recombination enzymes that function only with paired sectors of homologous DNA. But processes such as inversion, deletion, duplication, and translocation, often involving recombination between apparently non-homologous chromosomal regions, can alter the sequence of genetic information. The resulting chromosomal rearrangements have been observed in some cases with a disturbingly high frequency, inviting speculation about their biological significance. The processes involved have been termed collectively 'illegitimate recombination',[1,2] reflecting the bias for conventional pathways based on sequence homology; but evidence now accumulating may legitimise them as important mediators of evolution or even differentiation.

Classical geneticists have known for decades that eukaryotic chromosomes can behave unconventionally, giving rise to unexpected changes in phenotype. In the somatic tissue of plants or fruit flies, random spots of pigment arise against a background that is otherwise colourless, resulting in a mottled or variegated appearance. Closer examination of this phenomenon in maize led Barbara McClintock to the postulation that distinct genetic units—so called 'controlling elements'—are responsible for such changes in gene expression[3]. Drastic alterations of chromosome organisation, such as deletions, inversions and duplications, were often found in association with loci containing controlling elements[4]. Moreover, a given controlling element did not remain fixed at a particular site in the chromosome, but seemed to be capable of moving from one position to another, causing instability of gene expression and structural alterations at each new locus[5–9].

Thus controlling elements were revealed as possible agents of illegitimate recombination. But, as with many discoveries in classical genetics, interest in these elements was confined to a small group of scientists until molecular biologists succeeded, fifteen years later, in isolating analogous elements, the insertion sequences (IS), in prokaryotes[10–12].

The properties of IS-elements in bacteria and controlling elements in *Zea mays* have been described in detail elsewhere[9,13]. The aim of this article is to outline briefly what is known about bacterial insertion sequences at the molecular level, concentrating on the elements IS1 and IS2; to draw attention to parallels between the elements of eukaryotes and prokaryotes; to interpret data on eukaryotic controlling elements in the form of a molecular model; and to suggest what part these elements may play in evolution or differentiation.

IS elements in bacteria

Studies on the galactose and lactose operons of *Escherichia coli* revealed an unusual group of mutants that were shown conclusively to be caused by the insertion of a segment of DNA into the operon (for review, see ref. 13). When integrated in a gene of an operon, a DNA sequence of this kind not only abolishes the function of the gene, but also leads to a reduction in the activities of all other genes downstream in the direction of transcription, an effect known for historical reasons as polarity. (The term was originally used in connection with 'polar' mutations, in which the expression of genes downstream of the mutation is inhibited. Although it is now known that it is transcription that is polar, the mutations merely interfering with its progress, they are still said to 'cause polarity')[13–17]. The insertion sequences investigated so far fall into five non-homologous classes: IS1, IS2, IS3, IS4 and IS5 (for review, see ref. 18). The small size of these elements (800–1,400 nucleotide pairs), equivalent to only one or two genes, indicates that they differ from any known bacteriophage, and indeed, they seem to represent material displaced from the bacterial chromosome rather than invaders from without[19–21]. Eight copies of IS1 and five of IS2 are found as natural residents of wild-type *E.coli* chromosomes. Examination of their properties at 'unnatural' positions in the galactose and lactose operons has been conducted in order to gain information about their possible biological significance as components of wild-type chromosomes.

Polar effects of IS-sequences

The polar effects of insertion sequences have been shown to be due to interference at the level of transcription[22]. While the physical presence of an element in an operon causes some reduction in transcriptional read-through, a maximal block

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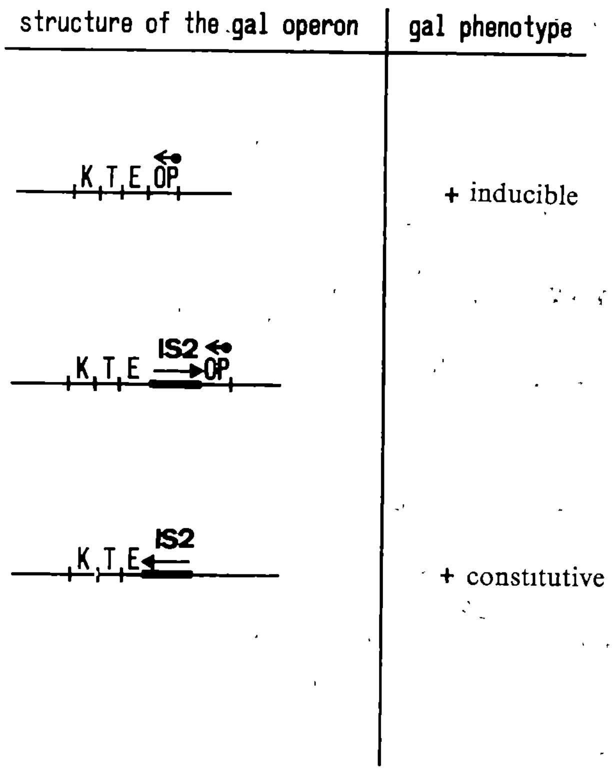


Fig. 1 Control of gene expression by IS2. Expression of the three genes of the galactose operon of *E. coli*—galactokinase (K), transferase (T), and epimerase (E)—before the insertion of IS2 is shown in line one. The genes are under normal operator/repressor control and expressed only in the presence of an appropriate inducer. Integration of IS2 into the operator region in polar orientation abolishes operon expression (line 2). Integration of IS2 in the opposite orientation leads to constitutive expression of the operon[38]. Long arrows indicate the orientation of IS2 and short arrows the direction of transcription from the operator region.

is achieved through the action of the bacterial protein rho[23-27]. According to a recent model of normal transcription termination in bacteria and phage[28], the rho protein recognises specific DNA sequences, the terminator sequences, at which it stops transcription. However, in order to be effective, rho must react with ribosome-free mRNA at these sites. This condition could be met if so-called 'nonsense' codons are situated immediately ahead of a terminator site. At these codons, for which no corresponding amino acids exist, translation ceases and ribosomes are released from the mRNA[29]. Thus it is possible that IS elements contain a combination of nonsense and terminator sequences which lead to rho dependent termination of transcription.

Excision of IS-elements

Wild-type revertants of IS-induced mutations, in which the IS-element is missing, can be found with a frequency of one in ten million[10,23]. They are thought to arise by exact excision of the IS element. Since normal function of the gene is restored, the integration and excision of the element must be possible without damage to the gene. But such precision is not always the rule, and in some cases, integration or excision is accompanied by deletions of bacterial material adjacent to the insertion site[13,30,31]. The enzymes involved in integration and excision have not yet been identified, but they are independent of known recombination enzymes, since both events occur in rec A hosts[14,32].

Transposition of bacterial DNA insertion elements

The excision of an IS sequence followed by re-integration of the element at another site is termed transposition. This process has been examined most extensively with transposons—elements closely related to IS sequences and which have been the subject of a recent review[33]. A transposon is a length of DNA embracing several genes and bordered at each end by identical sequences in reverse or direct orientation to each other. In a number of cases these termini consist of known IS sequences, while the intermittent material may include genes for resistance to antibiotics, positive markers which can be used to monitor the movements of the transposon. Transposons have thus been shown to move from the R (resistance) plasmid, in which they normally reside, to other plasmids such as the F (fertility) plasmid, to the bacterial chromosome, and to various bacteriophage DNAs as well (for review see ref. 33).

Transposition may be mediated by enzymes that are able to recognise the termini of insertion sequences and certain bacterial sequences. Alterations of these sequences, such as deletions, may influence the efficiency of transposition, as shown with the ampicillin transposon TnA (ref. 34). Transposition occurs in rec A hosts[35-37], which indicates that transposition enzymes are also independent of normal recombination functions. In one case, that of TnA, the transposon itself seems to code for transposition functions[34].

Control of gene expression by IS2

It seems that insertion sequences may be the components of a unique system of negative control, eliminating gene and operon activity on integration (see section on polar effects) and restoring it through precise excision. But from an evolutionary point of view a more interesting feature of IS elements may be their ability to implement positive control, as illustrated by the element IS2 (Fig. 1). While IS1 reduces operon expression when integrated in either direction, IS2 does so only when integrated in a particular orientation, orientation I. When it is in the opposite orientation, orientation II, genes located downstream are expressed constitutively at a level three times higher than normal[38]. This has been shown to be due to the presence on IS2 of a promoter at which transcription can be initiated[39]. This promoter may have other consequences when IS2 is integrated in orientation I, when transcription from the IS2 promotor may run head-on into an RNA polymerase coming from the existing promoter of the operon, thus contributing to the inhibitory effect of IS2 in orientation I (ref. 40).

The two antipodal effects of IS2 suggest that it can act as a simple switch. This is particularly appealing in view of evidence suggesting that 'flip-flop' control mechanisms underlie phenomena such as the alternating production of two different types of flagella in *Salmonella*[41-43] or the determination of mating type in *Schizosaccharomyces*[44]. An essential feature of such a switch mechanism would be the inversion *in situ* of IS2 or an equivalent element. There is genetic evidence for the inversion of IS2 in *E. coli*[38], although it has not yet been demonstrated by physical means. That inversion may represent a fundamental mode of regulation is shown by data from the bacteriophage mu. Inversion *in situ* of the so called 'G'-loop in this phage is clear both from genetic and from physical evidence[45,46].

IS sequences involved in chromosomal rearrangements

As striking as IS control of gene expression are the chromosomal rearrangements found in association with some insertion sequences. One prominent example is that of IS1-mediated deletion[31,47], illustrated in Fig. 6*a*. Sequences adjacent to this element are deleted with a frequency 10^2–10^3 times greater than the spontaneous frequency. In the cases investigated so far, IS1 is left behind in the chromosome during this process and continues to cause secondary deletions. IS1-mediated deletions all start at one terminus of the element and extend into the chromosome, ending at various non-random sites so that different classes of deletions occur with characteristic frequencies. Here too rec A-independent enzymes capable of recognising an IS terminus and certain chromosomal sites seem to be involved. Preliminary steps towards the identification of these enzymes have been made by the isolation of a bacterial mutant in which IS1-mediated deletion is reduced fortyfold[48]. Interestingly, the frequency of IS1 excision is not affected in the mutant, which suggests that different sets of enzymes are involved in these two processes.

Another type of rearrangement, duplication, can theoretically arise if chromosomal material is deleted in the form of a transposable structure which moves from one daughter strand to the other during replication. Duplications in association with an IS-element have been observed in mutants harbouring IS2 in the operator region of the galactose operon[49].

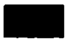
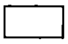
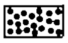
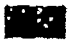
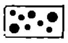
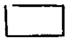
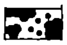
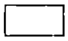
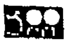
Controlling elements in *Zea mays*

In general, the effects of controlling elements in maize parallel those of DNA insertions in prokaryotes: integration of an element into a locus may produce a mutant phenotype, while accurate excision in some cells leads to clones in which normal expression of the locus is restored. If the locus at which an element is integrated contributes to a visible phenotype, such as pigmentation, occasional excision of the element during development results in a variegated appearance. In maize two kinds of element can be distinguished[9,50]: entities with no known gene products, referred to as receptor elements, and more complex, trans-acting units called regulatory elements. These elements can undergo integration, excision and transposition, for which specific enzymes may be postulated, as with bacterial DNA insertions. In maize, however, activities corresponding to such enzymes are supplied by the regulatory element itself, in a manner analogous to the bacterial TnA transposon (see section on transposition of DNA insertions). A regulatory element can thus move autonomously and govern the movements of receptor elements as well. The combination of a trans-active regulatory unit and a non-autonomous receptor element constitutes a two-element system, several of which have been found in maize, including the systems activator–dissociation (modulator), dotted, and Spm (enhancer).

These systems are entirely independent of each other, exhibiting no cross reactions between the regulator functions of one system and the elements of another. A detailed review of controlling elements in maize has been presented in a recent article[9].

For the following discussion we have selected Spm (suppressor–mutator), equivalent to En (enhancer) as a representative system, since it has some unusual properties not seen in other systems and has been the most thoroughly investigated. At this point it should be noted that most of the evidence for controlling elements has been obtained by classical genetic means. We shall waive a comprehensive presentation of this evidence and offer an interpretation on a molecular basis instead, in the hope of encouraging more widespread interest in this subject.

Fig. 2 States of the A1 locus in *Zea mays* and influence of Spm on gene expression. In wildtype kernels, in which no controlling element is present at the A1 locus (row 1), anthocyanin production and pigmentation are maximal and independent of the presence of Spm elsewhere in the genome. The degree of background colouration in all other kernels indicates the expression of the A1 locus as a function of Spm activity. Black spots correspond to clones in which a controlling element has been excised from the A1 locus under the influence of Spm mutator activity. The white spots on the kernel in row 9 are due to inactivation of Spm in two cells and their progeny. (For further explanation, see text).

phenotype of kernel	A_1 locus	state	Spm activity	location of Spm
	A_1		+ and −	not at A_1
	$a_1^{m}(-5)$		−	A_1
	$a_1^{m}(-5)$		+	A_1
	a_1^{m-1}	1	−	none
	a_1^{m-1}	1	+	not at A_1
	a_1^{m-1}	2	−	none
	a_1^{m-1}	2	+	not at A_1
	a_1^{m-2}	1	−	A_1
	a_1^{m-2}	1	+	A_1

Integration of an Spm element at the A_1 locus

The A_1 locus on chromosome 3 of *Zea mays* is one of a number of loci that contribute to anthocyanin production. As with most eukaryotic loci, the exact molecular nature of A_1 is unknown, but it may embrace several structural genes together with extensive control regions. Accordingly, various kinds of mutant can result, depending upon the position in the locus into which an element is inserted. For example, integration into a structural gene could lead either to total loss of the corresponding gene product or to loss of its distal end, resulting in a modified product. An element situated between a gene and its promoter may block transcription completely, allow a reduced level of expression, or have no effect at all.

In the mutants a_1^{m-5} (ref. 51) and the original a_1^m (ref. 52), the regulatory unit of the Spm system, referred to simply as Spm, is integrated in the A_1 locus. Anthocyanin production is eliminated in a_1^{m-5} and a_1^m kernels, but frequent, self-induced excision of the elements during plant growth results in pigmented sectors (Fig. 2, rows 2 and 3).

Trans-active functions of Spm

In the mutant a_1^{m-1}, as opposed to those discussed above, only the receptor element of the Spm system is incorporated in the A_1 locus[3]. In the original isolate of this mutant, expression of the A_1 locus is barely affected, and kernels exhibit a relatively high level of homogeneous pigmentation in the absence of Spm regulatory activity (Fig. 2, row 4). When an active Spm is present at some other position in the genome of a_1^{m-1} kernels, a different phenotype is produced. In these kernels, containing both elements of the Spm system, background coloration is abolished completely (Fig. 2, row 5). This reflects a unique property of the Spm regulatory element, which produces a suppressor activity that interacts with the receptor element at the A_1 locus to eliminate A_1 expression in the background tissue of the kernel. However, pigmented spots arise through excision of the receptor element from the A_1 locus under the influence of a second Spm activity, the mutator. The two trans-active Spm functions, suppressor and mutator, seem to be able to undergo mutation independently[53,54], which suggests that they are coded by separate genes on Spm.

States of a locus

In addition to mediating excision, the mutator product of Spm can induce inheritable alterations of the receptor element, leading to new 'states' of the locus in which it resides[54,55]. For example, exposure of the receptor element in a $^{m}_{1}{}^{-1}$ plants to Spm mutator activity leads to derivatives with new properties, representing different 'states' of the a_1^{m-1} locus[53,54] (see Fig. 2, rows 4–7). Some states derived from a_1^{m-1} show new levels of homogeneous pigmentation in the absence of Spm. Other states, derived from the allele, a_2^{m-1}, (ref. 56) respond to the suppressor but not the mutator activity of Spm, or vice versa. Still other, stable derivatives with a mutant phenotype respond

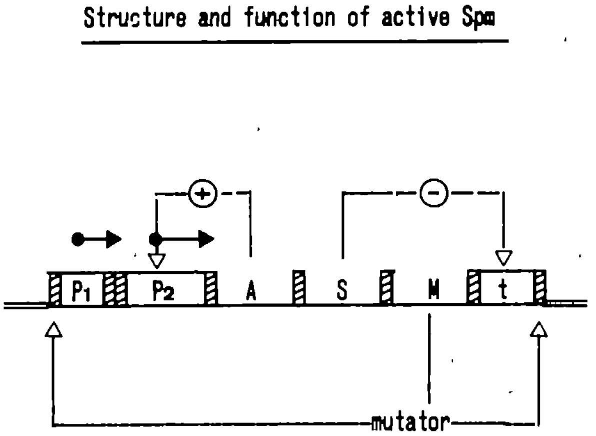


Fig. 3 Proposed structure and function of active Spm. A model of the molecular structure of an integrated Spm regulator element is illustrated. At one end of the element are two units, each with a promoter, P1 and P2. The direction of transcription from these promoters is indicated by the arrows above them. At the other end of Spm is a unit with a terminator sequence, t. Three structural genes are included in the element, coding for the A (activator), S (suppressor), and M (mutator) products. The action of these products is depicted by the dashed lines. The suppressor product operates at the t sequence to terminate transcription, as indicated by the symbol ○. The activator product, on the other hand, has a positive effect on transcription (○), by activating the P2 promoter. The mutator function of Spm is involved in excision of the element at the terminal sequences. So-called 'black box sequences', at which recombinational events such as inversion, deletions and duplications can occur, are represented by vertical hatched bars.

to neither Spm activity. One explanation is that new states arise through recombinational events such as inversions, deletions and duplications, or through conventional mutational events within the receptor element, altering its capacity to obstruct transcriptional read-through or the target sites for suppressor and mutator action. Short-range transposition of the receptor element within the locus may also be involved in some cases.

Transposition of controlling elements

A striking characteristic of controlling elements is their ability to move from site to site in the genome. Transposition of Spm, involving excision from one position and re-integration at another, is controlled by the mutator product of Spm, which seems to be effective only in the presence of suppressor activity[54]. Thus an active Spm can and does transpose itself autonomously, while an Spm that is inactive, as discussed below, remains fixed at one position.

While exicision of the receptor element of the Spm system is often seen, transposition has been reported in only one instance, that of the mutant wx_2^{m-8} (wx = waxy) derived from an a_2^{m-1} plant[57]. It should be noted, however, that transposition of the receptor element is common in other two-element systems in maize[3].

A model of Spm structure and functions

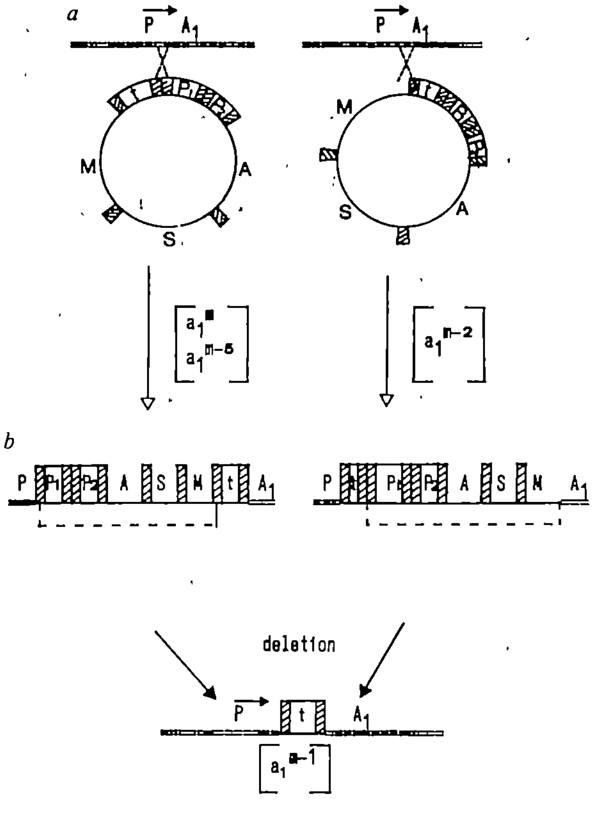
The repertoire of Spm activities is by no means exhausted by those described above. But before proceeding to a series of more subtle phenomena, such as cyclic changes in Spm activity and trans-activation, we wish to introduce a molecular model of Spm based on our knowledge of IS elements. It has been designed to fit a variety of observations into an intelligible framework and to promote discussion of new experiments.

The proposed molecular structure and functions of Spm are depicted in Fig. 3. The element includes three structural genes, one each for the suppressor and mutator products discussed above, and a third coding for a positive regulatory function, the activator, which will be discussed in the section dealing with trans-activation. There are two units analogous to IS2 at one end of the element, each with a promoter. At the other end is a unit similar to IS1, with a terminator sequence. Transcription can start spontaneously from P1, but transcription from P2 requires activation by the A gene product. The suppressor product of the S gene is envisaged as causing termination at the terminator sequence. The mutator product of the M gene executes excision at the indicated sites and induces alterations leading to new states, as discussed above. At the ends of Spm and interspersed throughout the element at various junctions are 'black box sequences', representing repeated sequences in reverse or direct orientation, comparable with those at the ends of bacterial transposons[37]. These sequences are not necessarily identical, but denote sites at which recombinational events such as inversions or deletions can occur.

Different modes of integration of Spm

By analogy with models of equivalent transposon processes, transposition of Spm can be considered as beginning with excision at the terminal sequences of the element, followed by circularisation and re-integration, as shown in Fig. 4(*a*). This process is catalysed by the mutator product. Integration at the site on the circular element between P1 and t gives mutants corresponding to a_1^{m-5} and a_1^m, in which the A locus is no longer expressed. Occasionally integration may take place at a different site on the circular intermediate, for example, the junction between M and t. This generates a mutant in which transcription started at the Spm promoter continues into the A1 locus. In this case, contrary to most situations described so far, the active element does not prohibit expression of the locus but promotes it. Obviously such mutants may be barely distinguishable phenotypically from wild type. However, if Spm is not transscribed, as in inactive phases described below, the mutant character is revealed, since no pigment is formed. This is exactly the case of the original mutant a_1^{m-2} (ref- 53) (Fig. 2, rows 8 and 9), which has been crucial to considerations of the molecular structure of Spm.

Fig. 4 *a*, Integration of Spm at the A1 locus in chromosome 3 of *Zea mays*, producing the mutant alleles a_1^{m-5}, a_1^m, and a_1^{m-2} whose phenotypes are depicted in Fig. 2. *b*, Possible origin of the receptor element from the alleles depicted in 4(*a*), leading to a two-element system.



Origin of the receptor element

Having established a model for Spm and mutants such as a_1^{m-5}/a_1^{m} and a_1^{m-2} caused by the integration of Spm into A1, let us now consider the origin of the receptor element in the Spm system. In a number of cases [7,51,52,53], a mutant harbouring a single, autonomous regulatory element at a given locus has generated derivatives in which only the receptor element is found at the locus, apparently accompanied by transposition of the regulatory element to another position. An explanation for this observation, which has been discussed elsewhere[58], is elaborated in Fig. 4(*b*).

When part of an integrated Spm is deleted, perhaps through inaccurate excision, the t unit may be left behind as a fragment that is still responsive to Spm, since it contains the target sites for both the suppressor and mutator functions. This is the situation found in the mutant a_1^{m-1} discussed earlier. When this residual unit is located between an A_1 structural gene and its promoter, transcriptional read-through may be reduced to some extent. However, when suppressor product is supplied by an active Spm, it acts at the terminator sequence of the receptor element to block transcription completely, just as the bacterial rho protein does at IS-sequences. The original level of expression of the locus can be restored when the receptor element is excised.

In addition to producing a receptor element corresponding to a complete t unit, as discussed above, partial deletion of Spm could also leave behind fragments that are greater or smaller than the t unit, resulting in various different states of the locus[8].

It is especially significant that the exceptional mutant a_1^{m-2} can generate a two-element system with a phenotype similar to the a_1^{m-1} phenotype described above[53]. This lends support to the proposal that the regulatory element in a_1^{m-2} mutants contains the same components as in other mutants such as a_1^{m-5} but in a different order, as shown in Fig. 4(*a*).

Cyclic changes in activity of Spm

An interesting property of Spm is its tendency to become inactive[54,59]. Inactive phases alternate with periods of activity with a characteristic frequency. Since an inactive element, expressing neither of its two products, occasionally becomes reactivated, these products do not seem to participate in such an activity change[54,60].

The effector of cyclic activity changes must be sought elsewhere, as shown by mutant alleles such as $a_1^{m(flow)}$ (ref. 59), which exhibit Spm activity only in certain parts of the kernel. The mutant $a_1^{m(flow)}$, has an Spm subject to activity changes at the A1 locus. In kernels of this mutant Spm is active only within a limited area at the base of the kernel. The element in the $a_1^{m(flow)}$ mutant is apparently sensitive to activating signals produced during the development of the base of the kernel.

In the terms of the molecular model of Spm, cyclic activity changes may result from inversion *in situ* of the promoters under the influence of extraneous signals, as in the inversion of IS2 discussed earlier.

Inversion of one or both promoters produces three different inactive structures, shown in Fig. 5. The result of inverting both promoters (Fig. 5*a*) is obvious. When only P2 is inverted, as in Fig. 5(*b*), RNA polymerase can still initiate transcription at the P1 promoter, but it will be obstructed if it collides with transcription machinery from the P2 promoter (see section on polar effects of IS2). When only P1 is inverted, as in Fig. 5(*c*), transcription of Spm from P2 can continue as long as activator product is present in the cell. The element will become inactive only when activator product in the cell has been sufficiently diluted. Because of this dilution requirement, it is unlikely that inversion of P1 is involved in somatic activity changes that occur during the development of a kernel. But an inactive structure of the kind shown in Fig. 5(*c*), is important for considerations of trans-activation, discussed in the next section.

Through the action of mutator product, an Spm regulatory element that exhibits a certain frequency of cyclic activity changes can mutate to alternate with a new frequency[60]. This indicates that something within the element itself determines the frequency (and time) of activity changes. These determinants are represented by the 'black box' sequences shown in the model in Fig. 3, at which inversion might occur. Mutational alteration of these sequences could affect the frequency of inversion.

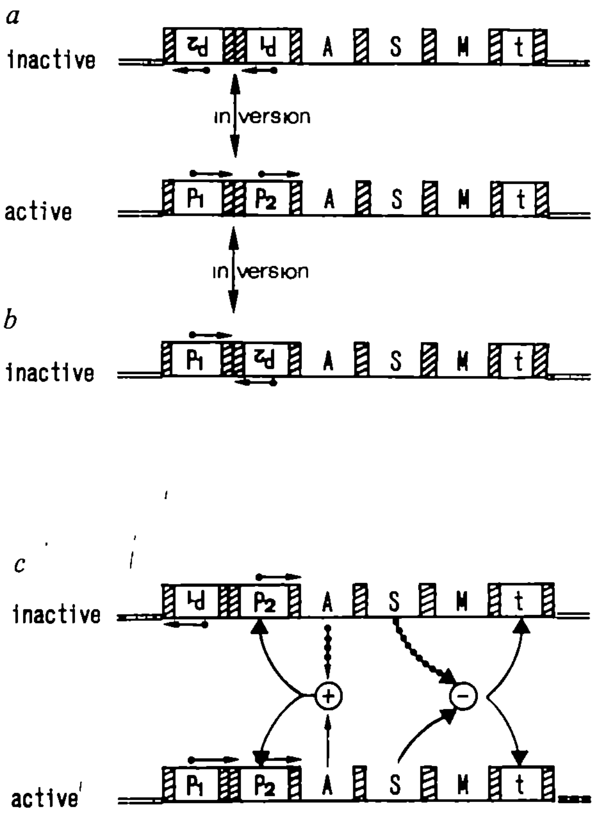


Fig. 5 Cyclic changes in activity of Spm. Inversion of one or both Spm promoters leads to three different inactive structures (*a*, *b* and *c*). The structure illustrated in *c* can be trans-activated when activator product is supplied by an active Spm.

Trans-activation of Spm

In a key experiment it has been demonstrated that an inactive Spm can be temporarily activated when a second fully functional Spm is introduced into the genome[60]. This was detected in a unique set of conditions which made possible phenotypic discrimination between kernels with double Spm activity and those with only one dose.

Normally when a receptor element is integrated at a locus, the same phenotype is produced regardless of whether one or more Spms are present in the genome. In one experiment, however, a special allele of a_2^{m-1} was used to test for Spm activity[55]. In plants with this allele the receptor element at the A_2 locus allows some pigment production when Spm is absent. In the presence of Spm suppressor product expression of the locus is eliminated and no pigment is produced. The unusual property of this receptor element is that it can no longer be excised in response to the mutator product. Thus in such a_2^{m-1} kernels, pigment is produced only in sectors in which Spm is missing or inactive; when two Spm are present, pigment is produced only when both are inactive. Since two inactivation events are less probable than one, a_2^{m-1} kernels with two Spms have fewer pigmented spots than those with one Spm.

With kernels containing the special a_2^{m-1} allele together with an active Spm, and one that is usually inactive for a longer period of time, one would expect to see a pattern of pigmented spots characteristic of a single, active Spm. But, surprisingly, kernels with a double dose phenotype are obtained[55], reflecting

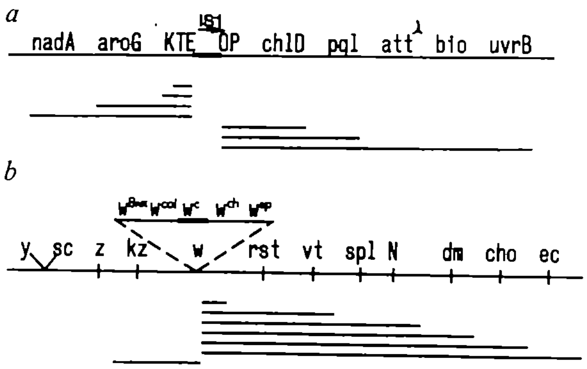


Fig. 6 *a*, Deletions induced by IS1 in the galactose operon of *E. coli* and (*b*) by the controlling element in the white locus of *D. melanogaster*. Data taken from refs. 47 and 64. Horizontal bars indicate extent of material removed in various classes of deletion derivatives

two active Spms. The originally inactive element is not turned on permanently in this process, however, and ceases to be expressed when its active counterpart is removed by meiotic segregation.

The observation of trans-activation implies a number of features of Spm, which have been incorporated in the model shown in Fig. 5(*c*). First, the temporary nature of the trans-activation suggests that it does not involve structural alteration of the inactive element. It follows that the S gene and a promoter for the S gene represented by P2 must be arranged in potentially transcribable orientation within the inactive element. The inactive condition of this element can then be accounted for by assuming that P2 is inactive unless stimulated by activator product, supplied in this case by an active Spm. For normal expression of Spm, a second promoter, P1 is proposed, from which transcription occurs spontaneously, leading to expression of the A gene. In addition, the double dose phenotype observed during transactivation indicates that each element is inactivated independently to produce pigmented spots. From this observation it can also be deduced that both the A gene and the S gene are situated downstream from P1 and P2, and that expression of the A gene triggers an autocatalytic cycle of expression of Spm.

Chromosomal rearrangements involving controlling elements

In the preceding sections we have summarised how the integration, excision, and transposition of elements of the Spm system and the action of certain Spm products function to control the expression of some loci in maize. But controlling elements, like IS sequences, have also been shown to enhance the incidence of inversions, deletions, and duplications of adjacent chromosomal regions, although much less attention has been devoted to this phenomenon. In maize, rearrangements of this kind have been observed in particular with the element dissociation[4]. There is evidence that elements resembling IS elements also occur in *Drosophila*[61,62], where they are frequently associated with chromosomal rearrangements. Detailed analysis of such processes has been facilitated by the many well-mapped genetic markers of this organism and by the banding patterns of its salivary gland chromosomes which permit visual detection of structural alterations. In one case, a transposable element seems to have been involved in a duplication of part of the white locus for eye colour[63]. An even more striking example is that of deletions found in association with another controlling element in the white locus of white–crimson mutants (Fig. 6*b*); in direct analogy to IS1 mediated deletion in bacteria, these deletions all start at the white locus and extend in either direction, ending at non-random points in the chromosome, resulting in distinct classes of deletion[64]. In this process the controlling element, like IS1, is retained in the deletion derivative where it can cause a second round of deletion[61].

The prevalence of IS sequences and controlling elements in organisms as diverse as *E. coli*, *Zea mays* and *Drosophila* suggests that they may be of general biological significance. There are, moreover, hundreds of other organisms that possess 'mutable genes', and exhibit phenotypes similar to those caused by controlling elements (see ref. 9). Furthermore, we have already noted that IS-sequences are natural residents of the wild-type *E. coli* chromosome, and there is evidence that controlling elements also exist at unknown positions of wild-type maize chromosomes, from which they can be 'dislodged' by irradiation[65], or mechanical disruption of the chromosome[4,5,66]. Whether they exert control functions at these positions or are simply kept in reserve as prefabricated units for the evolution of new control circuits remains unclear.

The large scale rearrangements found in association with such elements provide interesting grounds for speculation. Duplication, for example, is regarded by many as an indispensable process in evolution, producing a copy of genetic information with which mutational experiments can be conducted at lower risk and exempt from immediate selective pressure[67,68]. Inversions, deletions, and transpositions, on the other hand, may serve to create new nucleotide sequences or even new genes at fusion points. These processes may also represent important evolutionary means for reshuffling genetic information, uncoupling genes from old regulatory signals and linking them to new ones. This would provide adaptive flexibility while permitting conservation of vital or successfully evolved genetic information. Sequences analogous to IS sequences or their termini may serve as substrates for recombinational events leading to such rearrangements.

One recent observation suggests that chromosomal rearrangements may also play a part in differentiation. The variable and constant parts of the immunoglobin light chain seem to be coded by two different genes that are fused and expressed as a joint polypeptide[69]. In embryonic cells these genes seem to be separated, since they are located on different DNA restriction fragments. But in certain myeloma cells, which are thought to represent advanced developmental stages, arrested at a certain point, the two genes are found on the same DNA fragment. This has been interpreted to mean that fusion of these genes occurs curing development through deletion of the intermittent material, inversion, or transposition.

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articles

Protein–DNA and protein–hormone interactions in prealbumin: a model of the thyroid hormone nuclear receptor?

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High resolution X-ray analysis of the hormone-binding protein prealbumin has shown that it has a structural complementarity to double-helical DNA. The proposed binding site is composed of two symmetry-related β-sheets containing a pair of helically disposed arms, which can interact with the bases in the wide groove of DNA. A palindromic target sequence is indicated by the symmetry of the protein. The two identical thyroid hormone binding sites on prealbumin are located in a channel that runs completely through the molecule. These two structural features suggest prealbumin as a model for the thyroid hormone nuclear receptor, providing a number of detailed predictions of its properties.

The generally accepted model for the expression of hormone activity gives a central role to the hormone receptor. Receptors are presumed to be proteins that have two essential properties: the ability to form extremely tight and specific complexes with particular hormones, and the ability to convert the interaction into a signal that initiates the response of the target cell. Although the receptors for many hormones are located in the outer membrane of the target cell, there is increasing evidence that at least one class of receptor for the thyroid hormones is located in the cell nucleus[1-4]. Since these hormones exert their effects primarily on gene expression, it seems probable that their receptors may be able to initiate this response through binding to the double-helical DNA in chromatin, but little is known of their mode of action at the molecular level.

The thyroid hormones are water-insoluble molecules that require specific binding proteins in the plasma and the cell cytosol to enable them to gain access to the nuclear receptors. In an attempt to define the nature of the interactions between the thyroid hormones and their binding proteins, we have been working on the X-ray structure of prealbumin, one of the two thyroxine-binding proteins in human plasma. Prealbumin has a molecular weight of 55,000 and is composed of four identical subunits of 127 amino acid residues[8], arranged with tetrahedral symmetry[5]. The protein has two hormone binding sites whose association constants[9] for L-thyroxine (T_4) are 1.05×10^8 M^{-1} and 9.55×10^5 M^{-1}; triiodo-L-thyronine also binds at these sites about an order of magnitude less strongly. The X-ray results indicate that these two sites are structually identical and are deeply buried in a narrow cylindrical channel that runs through the centre of the protein molecule. Prealbumin also carries independent binding sites for retinol-binding protein (RBP) (ref. 10), the specific plasma carrier of vitamin A. There are apparently four equivalent binding sites for RBP (ref.11), but information on their location and interactions is at present lacking.

We have now discovered, however, that part of the surface of the prealbumin molecule is complementary in size and shape to the stable B-form of double-helical DNA. This putative DNA-binding site, which has the form of a deep semi-cylindrical groove equipped with a pair of helically disposed arms, is constructed almost entirely from a symmetry-related pair of β-sheets. Although in principle the great diversity of protein structure allows binding sites for any ligand to be constructed, no detailed model of an interaction between native, double helical DNA and a globular protein has yet been proposed. The site we observe is almost entirely composed of regular protein structure, and therefore provides a general model for protein–DNA interactions, especially as the mode of binding that is indicated incorporates many of the features that have been proposed for such interactions[12].

Because prealbumin has no known function that may involve DNA binding, nor as a soluble plasma protein is there any compelling reason to anticipate such a function, the significance of the structural complementarity to DNA is not immediately obvious. In particular, we cannot rule out the possibility that the site is merely a coincidental feature of the molecular structure, without biological significance. But the closeness of fit and the mode of interaction with the standard DNA double helix suggest that we should seriously consider the alternative possibilities that either prealbumin has so far undetected DNA-binding function, or that it is related to a protein that does have such a function. Of these possibilities the first can be tested either by careful model building of the proposed prealbumin-DNA complex or by direct binding studies, while the second can only be approached at present by speculative arguments. Nevertheless we feel justified in considering the second possibility in more detail because, as the one known protein whose function combines both thyroid hormone and DNA binding, the thyroid hormone nuclear receptor is the most obvious potential relative of prealbumin, implying that the interactions we observe in prealbumin may shed some light on the properties of the receptor itself about which little is otherwise known. These considerations lead us to interpret the presence of a DNA binding site on prealbumin as indicating the possibility that some or all of the thyroid hormone binding proteins belong to a family whose members have evolved from a common ancestor, and in particular that prealbumin and the receptor have evolved from an ancestral protein whose structure encompassed both hormone and DNA binding sites. Evidence for a relationship between prealbumin and the other known thyroid hormone binding proteins (the receptor and the plasma protein, thyroxine-binding globulin, TBG) is limited by our lack of knowledge of the characteristics of these proteins, especially of their amino acid sequence. The only known properties of the receptor, its molecular weight[13] and the general chemistry of its hormone binding site [14], are similar to prealbumin; while prealbumin and TBG share the same molecular weight, subunit structure, single strong T_4 binding site per tetramer, and the same extreme stability towards denaturants[15]. Although this evidence is circumstantial and meagre, it does lend some support to the proposal. We therefore present the details of the protein–hormone and protein–DNA interactions in prealbumin for consideration as a model of the thyroid hormone nuclear receptor. The model predicts a number of verifiable properties of the receptor, including its molecular properties, the nature of its hormone and DNA binding sites, and a possible pathway of interaction between them.

X-ray analysis

A preliminary description of the tertiary and quaternary structure of the prealbumin molecule determined from a 2.5-Å resolution Fourier map phased with three isomorphous derivatives has been reported[16]. Following this preliminary analysis the complete amino acid sequence of prealbumin has been determined[8], and readily incorporated into the X-ray model. This model has now been partially refined against 1.8-Å resolution X-ray data—the practical limit of the diffraction pattern—to give an overall R factor of 29% for the 24,000 unique reflections, where

$$R = \frac{\Sigma \,\|F_{\mathrm{obs}}| - |F_{\mathrm{calc}}\|}{\Sigma\, |F_{\mathrm{obs}}|} \times 100$$

Although the refinement is being continued, the present stage enables us to give a reliable picture of the molecule and its binding sites.

The prealbumin monomer is a classic 'β-barrel' molecule, with eight β strands organised into two four-stranded sheets. The whole molecule is a tetramer with subunits arranged tetrahedrally. The β sheets represent the major points of subunit interaction: monomers associate into dimers by antiparallel hydrogen bond interactions between equivalent sheets giving the dimers two eight-stranded sheets; the dimers associate in the whole molecule by opposing equivalent eight-stranded sheets face-to-face in the centre of the molecule. The prealbumin molecule shown in Fig. 1 is therefore a very simple structure composed of four eight-stranded β sheets—an 'inner' pair sandwiched between an 'outer' pair. A remarkable feature of the structure is that the inner sheets are not in contact but form, or are separated by, an open channel that runs through the molecule.

Low resolution X-ray analysis[11] of prealbumin complexed with its hormone ligands, T_3 and T_4, has shown that they are located at two symmetry equivalent sites located deeply within the central channel. As with some other proteins, it has proved difficult with prealbumin to define conditions in which the ligands will bind reliably in the crystals. We suspect the reason for this is the very low solubility of the hormones in the mother liquor of crystallisation, but whatever the reason, we have so far been unable to collect the high resolution three-dimensional X-ray data that is needed fully to define the nature of the

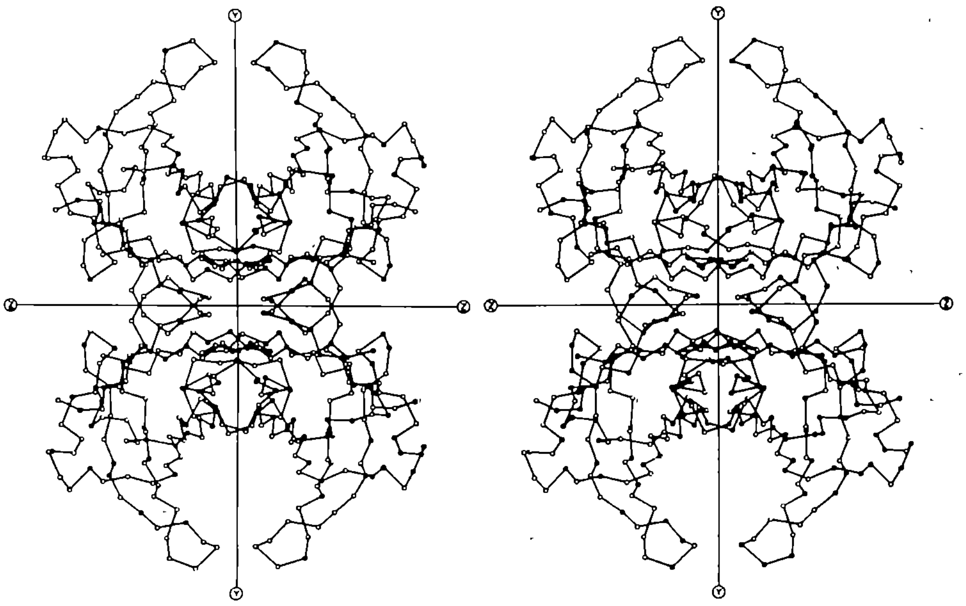


Fig. 1 A stereo drawing of the α-carbon positions of the prealbumin tetramer looking down the molecular x axis. The hormone binding sites are located in the channel that runs horizontally through the molecule parallel to the z axis. The proposed DNA binding sites are located at the top and bottom of the figure, so that the DNA is located with its helix axis parallel to x.

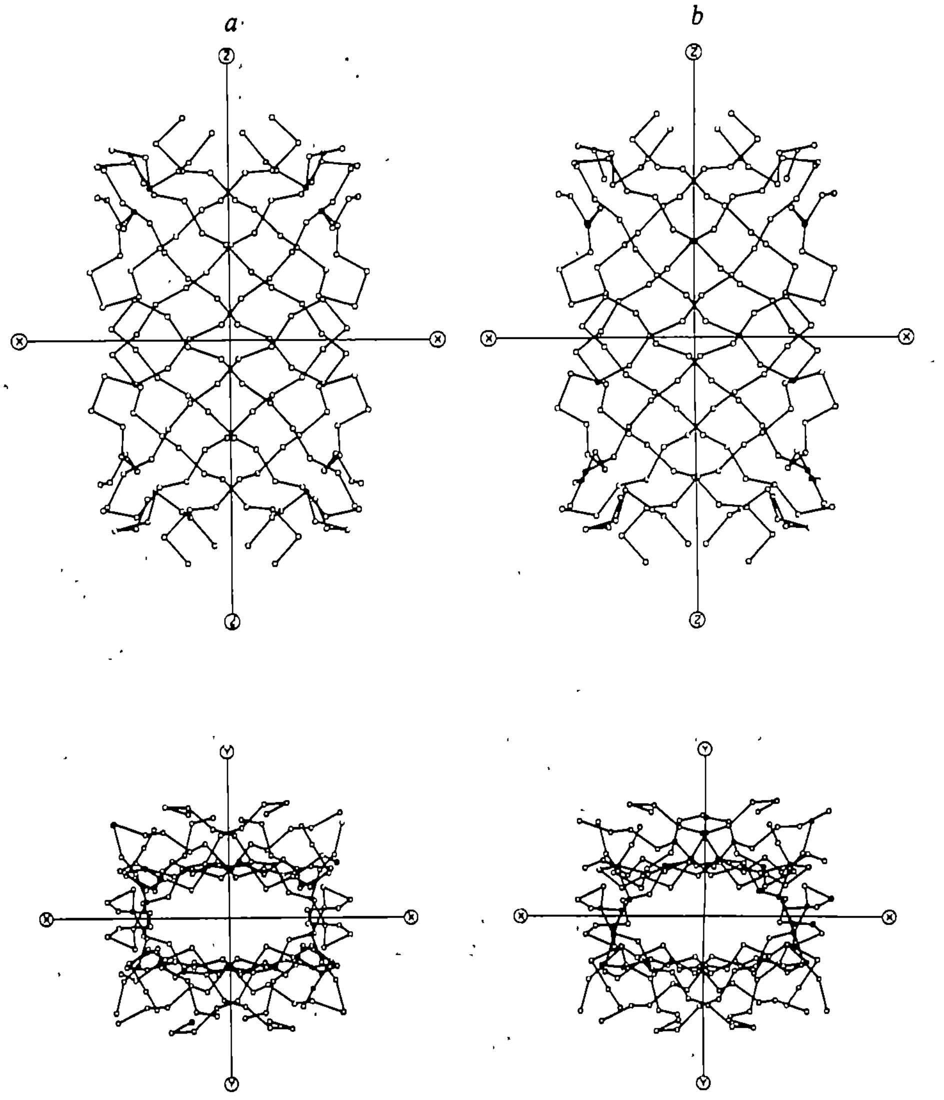


Fig. 2 Stereo diagrams showing the organisation of the polypeptide chains of the four subunits around the central channel; *a*, looking down the *y* axis; *b*, looking down the *z* axis. The circles represent carbon positions.

protein interactions. However, we have been able to collect high resolution data in projection that seem to give results fully consistent with the previous low resolution three-dimensional maps.

The thyroxine binding site

The central channel that contains the thyroxine binding sites can be seen in Fig. 1 running horizontally through the centre of the molecule. Stereo views of the local structure of the channel are shown in Fig. 2, looking down the *y* axis and the *z* axis (the axis of the channel). The surface of the channel is tightly organised from the polypeptide chains of the inner, four stranded β sheet of each of the four subunits, and takes the form of a 16-stranded β cylinder about 50 Å long. The molecular 222 symmetry divides the channel into two symmetry-related halves clearly showing that the two binding sites are identical. In addition one of the molecular twofold axes coincides with the channel axis showing that each site itself has twofold symmetry. Figure 3 shows one half of the channel with the side chains that project into it. The chemistry of the channel is characterised by three elements arranged linearly along it, which reading from the centre are: (1) a hydrophilic patch formed by the hydroxyls of the Ser 117 and Thr 119 pairs of residues; (2) a hydrophobic patch formed by the methyl groups of the Leu 17, Thr 106, Ala 108, Leu 110, Thr 119 and Val 121 pairs; and (3) a group of charged residues including the paired side-chains of Lys 15, Glu 54 and His 56. The absence of any of the molecule's 48 hydrophobic aromatic residues from the channel is both clear and striking. An analysis of the free space around the axis of the channel was carried out by calculating van der Waals cross sections in 1-Å thick sections along its length. The analysis showed that there is a cylindrical hole along its length with a fairly constant diameter of about 8 Å, except at two symmetry-related positions some 4 Å on either side of the centre of the channel where the close approach of the paired side chains of Leu 110, Ser 115 and Ser 117 cause a marked local constriction.

The low resolution X-ray analysis of complexes of prealbumin with both T_3 and T_4, showed that the hormones are bound exclusively at two symmetry-related sites buried deeply within the central channel (see Fig. 8 of ref. 16). The difference electron-density envelopes for both ligands were elongated along the axis of the channel and extended from about 4 Å from the centre to about 16 Å. Interpretation of these features suggest that the two hormones are bound in much the same way, located between the constriction near the Ser 117–Thr 119 pairs of side chains and the Lys 15–Glu 54 pairs of charged residues, with their long axes more or less parallel with the axis of the channel. The identity of the two binding sites by symmetry confirms the proposal[8,17] that the difference in binding constants implies negative co-operativity between the sites. The constriction observed near the centre of the channel would prevent access from one binding site to the other, indicating that the interactions are of the site–site rather than ligand–ligand type (compare refs 9 and 17).

The 3-Å resolution projection maps of the ligands have been used to attempt to locate the iodine substituents of the hormones within the overall electron-density envelopes observed in the low resolution maps. Although fully self-consistent results have been obtained, projection maps of proteins can be very misleading and the interpretations given below must be regarded

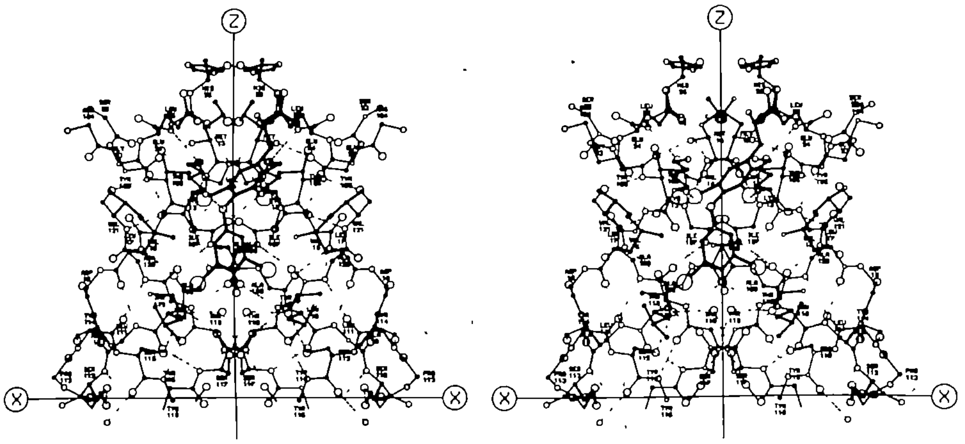
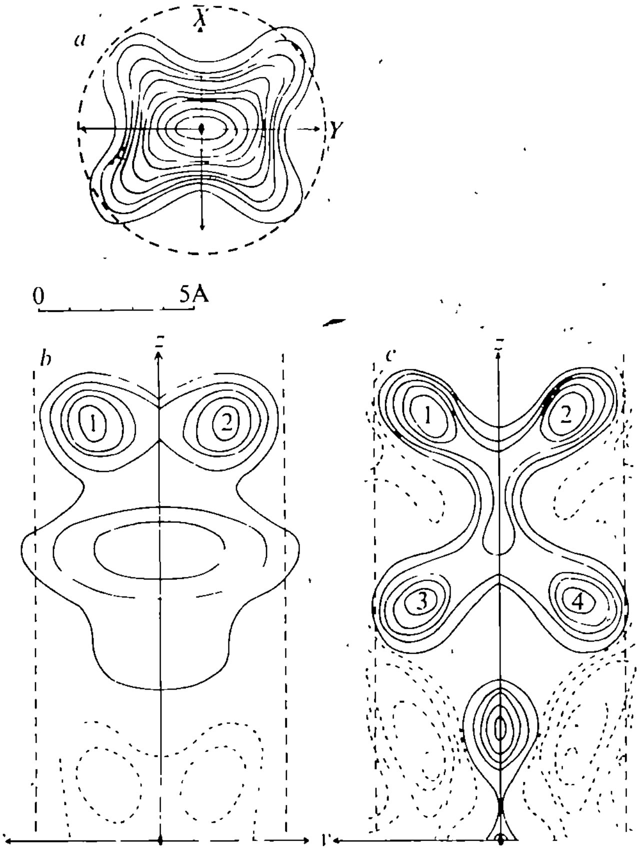


Fig. 3 Stereo diagram of the atomic arrangement in a single hormone binding site. This drawing corresponds to the upper half of Fig. 2(*a*). The main chain is shown in light line and side chains that point into the site are shown in medium line. The position of the T_4 molecule, shown in heavy line, was derived by fitting the coordinates of Cody[14] to the iodine positions indicated in the projection maps.

as provisional until three-dimensional high resolution data on ligand binding is obtained. The difference map of T_3 binding projected down the *z* axis (the axis of the channel and approximately the long axis of the hormone) is shown in Fig. 4*a*. It can be interpreted as indicating that the benzenoid rings of the hormone are roughly at right angles as expected (the bulky iodine substituents cause other orientations to be energetically unfavourable), and are orientated at about 45° to the molecular *x* and *y* axes. The equivalent maps of T_3 and T_4 projected down the *x* axis shown in Figs 4*b* and *c* contain discrete peaks consistent with Fig. 4*a*, that can be interpreted as representing the various iodine substituents of the hormones. It can be seen that the pairs of peaks labelled 1 and 2 further from the centre of the channel are coincident in both maps, but that the inner pair labelled 3 and 4 in the T_4 map is replaced by a diffuse region of density in T_3. The simplest interpretation of the two maps is that the common pair of peaks represent the 3,5 pair of iodines in each hormone, that the inner pair of peaks in T_4 represent its 3′,5′ pair of iodines, and that the diffuse density in T_3 represents alternative positions of its single 3′-iodine.

Fig. 4 3-Å resolution difference maps of the binding of (*a*) T_3 projected down the *z* axis; (*b*) and (*c*) T_3 and T_4 respectively, projected down the *x* axis. Contours are at equal but arbitrary levels; positive in full line, negative in broken line. The confines of the channel, assuming a constant diameter of 8Å, are shown in heavy broken line.



Taking the interpretations of the projection maps together it is possible to define a tetrahedron of iodine substituents for T_4 that is generally consistent in size and geometry with that found in the structures of the crystalline hormone[18,19]. However, to obtain the points of interaction between the iodines and the protein, an ambiguity concerning the orientation of the tetrahedron—are the peaks 1 and 4 of Fig. 4*c* above the plane of the projection and peaks 2 and 3 below, or vice versa—has to be resolved. A provisional solution has been achieved by finding that one orientation can be fitted in the channel without producing unacceptably close contacts with the protein, but that the other cannot. Assuming the former orientation to be correct (shown in Fig. 3) each pair of iodines can make favourable hydrophobic contacts with protein side-chains. Each of the 3′,5′-iodines lies between and in contact with the side chains of Leu 17 and Leu 110, and each of the 3, 5-iodines fits into a pocket lined with the methyl groups of Thr 106, Ala 108 and Val 121 and the β- and γ-methylenes of Lys 15. These positions are also consistent with the interaction of the hormone's 4′-hydroxyl, through a well defined water molecule, with the hydroxyls of the Ser 117 and Thr 119 pairs of residues in the hydrophilic patch, and with the interaction of the hormone's α-carboxylate and α-amino group with Lys 15 and Glu 54 respectively. This latter interaction, and with it the broad outline of this proposal for hormone binding, is strongly supported by affinity labelling experiments on prealbumin using dansyl chloride[20] (a competitive inhibitor of T_4) and bromoacetyl-T_4 (ref. 21), both of which have been found covalently attached to Lys 15.

The DNA binding site

Our attention was drawn to the possibility of a DNA-binding site on prealbumin by the remarkable cylindrical–helical depression in the outer surface of the molecule, shown in Fig. 1. This site is formed by the 'outer' β sheets of two symmetry-related subunits. The normal right-handed twist of the sheets coupled with the symmetry induced by the molecular twofold axis parallel to *y* gives rise naturally to a concave cylindrical structure, that is given its helical character by the extended hairpin loops made by the two outer strands of each sheet. The structure so formed has a length of about 40 Å, a diameter of about 25 Å and a helical pitch of about 33 Å.

The extent to which this site is complementary to double-helical DNA was explored by model building, using an interactive computer display system[22]. The coordinates used were those of the main-chain atoms of prealbumin from the latest stage of refinement, and a standard set for all the atoms of the B form of the DNA double helix[23]. These coordinates were maintained throughout the fitting, which therefore corresponded

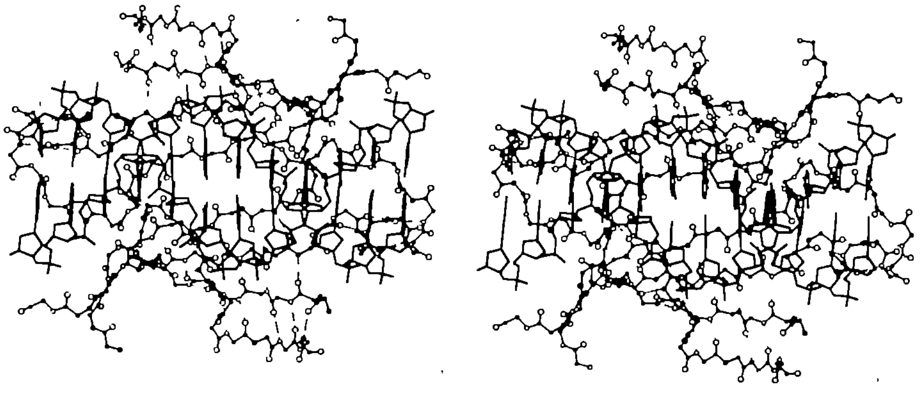


Fig. 5 Stereo diagram of the proposed prealbumin–DNA complex looking down the *y* axis, with the *x* and *z* axes horizontal and vertical respectively. The main chain atoms of the outer β-sheet are shown in light line with the hydrogen bonds in broken line. The DNA is drawn in heavy line. All the atoms in the diagram are related by the twofold axis parallel to *y* that is positioned at the centre of the diagram.

to the mating of two rigid molecules. The first step in locating the DNA in the site was carried out by making the molecular *y* axis of the protein coincide with one of the twofold axes of the DNA helix that runs between each base pair perpendicular to the helix axis. When the protein and DNA twofold symmetry axes coincide, only two degrees of freedom remain: rotation of the DNA around the axis relative to the protein, and translation of the DNA along the axis relative to the protein. Rotation of the DNA around the axis was seriously restricted by the helical arms to a narrow range of orientations in which the DNA helix runs closely parallel to the axis of the cylindrical channel. In this orientation the helical arms of the protein fit closely into the wide groove of the DNA double helix. A rotation of 180° from this orientation—the only alternative position—is not as favourable because the helical arms are too large to fit into the narrow groove of the DNA. Translational adjustment of the DNA along the common symmetry axis was relatively easy because it corresponds to bedding the DNA down the channel, making due allowance for the protein side chains.

The final fit of the DNA to prealbumin obtained from the computer display is shown in Figs 5 and 6. Its characteristics are as follows.

(1) The twofold axis of symmetry of the protein coincides with the twofold axis of symmetry in the DNA.

(2) The DNA double helix lies in the pronounced cylindrical depression in the protein molecule with its helix axis parallel to the axis of the depression.

(3) The pair of helical arms of the protein fit closely into the wide groove of the DNA double helix. This is the most striking feature of the fit because it could allow the arms to interact with the DNA bases whose edges are exposed in the wide groove. We therefore consider that the helical arms can act as 'reading heads' that probe the DNA for a compatible base sequence. The twofold symmetry relation of the arms would necessarily imply that such compatible base sequences must be palindromic. But Fig. 5 shows that this is not necessarily true for the two base pairs at the very centre of the binding site which are in fact more distant from the protein surface than the base pairs on either side. This relieves the requirement for an exact palindrome at the centre of the interaction site as has been observed for the *lac* operator[24] and the *Haemophilus* endonuclease specificity site[20].

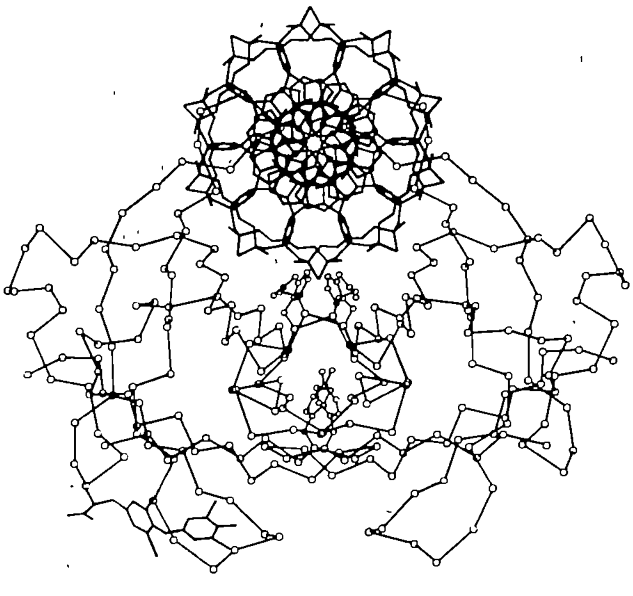
We have not yet carried out the careful model building of DNA in the proposed site on prealbumin that might tell us whether the protein, in addition to having an appropriate structure, also has the appropriate side chain distribution for favourable interaction with DNA. At present, therefore, we can only note that the proposed binding site on the protein is especially rich in ionic side chains; ten lysines, four histidines, eight glutamates and six aspartates are located in positions where they could interact with some part of the DNA. In addition Trp 41 is located in a totally exposed position on the helical arms with its indole ring parallel to and between neighbouring base pairs, suggesting that intercalation should be considered in any possible scheme of interaction.

Finally, any model of a hormone nuclear receptor should indicate the possibility of a means of communication between the hormone and DNA-binding sites. That some structural response to thyroxine binding takes place in the prealbumin molecule is indicated by the negative cooperativity of binding. The nature of this structural response, which may alter the geometry and properties of the DNA-binding site, can be determined by high resolution X-ray studies of hormone binding. In the absence of these studies we can only note that the separation and orientation of the symmetry-related pairs of β-sheets that form the DNA site, could be controlled by Tyr 116 (whose position is shown in Fig. 6) whose hydroxyl is inserted between the inner strands of the two β-sheets, forming a hydrogen-bond bridge between them. The residue following this tyrosine, Ser 117, is located in the hormone binding site and is hydrogen bonded through a water molecule to the 4′-OH of thyroxine. Although there may be other possibilities, Tyr 116 is the most obvious means of communication between the sites, because it is uniquely situated with its peptide involved in the β-structure of the hormone binding site and its hydroxyl involved in the hydrogen bonding network of the β-sheets that form the DNA binding site.

Conclusions and predictions

The most important general conclusion from this work is that the typical binding site depression of a globular protein molecule

Fig. 6 A drawing of the proposed prealbumin–DNA complex looking down the *x* axis, with the *y* and *z* axes vertical and horizontal respectively (compare with Fig. 1). The main chain of the prealbumin dimer is shown in light line; the circles represent the α-carbon positions. The symmetry-related pair of tyrosines 116 are shown at the lower centre, and two pairs of side chains that point into the DNA binding site are shown in the centre. The DNA double helix is seen end-on in heavy line at the top, and one T_4 molecule in its (half) site is drawn in heavy line at the lower left.



can take on the helical character necessary for combination with native DNA when the binding region is composed of a symmetry-related pair of β-sheets. If the outer strands of the sheets 'peel off' from the body of the protein, as occurs in prealbumin and also, in different contexts, in a number of other β-structured proteins, a pair of helically-disposed 'arms' are formed that can fit into the grooves of the DNA double helix where they can probe the nucleotide bases for a complementary sequence. As a consequence of the twofold symmetry of the site, the complementary base sequence must necessarily correspond either to an exact palindrome or alternatively to a palindrome with a gap at its centre. The latter case arises because the arms fit the outer parts of the target sequence more closely than its centre. Although the particular structure of prealbumin suggests a potential interaction with a six or eight base palindrome, it can easily be extended to provide a general basis for protein–DNA interaction, that can account for the four-base palindromic target sequences of some restriction enzymes[26–28] and the 20-base 'gapped' palindrome of the *lac* operator[24]. Details of this general model will be published elsewhere.

In prealbumin the thyroxine binding sites are located deeply inside a cylindrical channel that runs completely through the molecule. When bound at these sites the hormone is almost completely removed from the surrounding medium. The binding sites have twofold axial symmetry to which the hormone is surprisingly well-suited and provide individually favourable microenvironments for each of the characteristic substituents of the hormone. Minor side-chain substitutions in a site of this kind, such as possibly the inclusion of aromatic residues at the iodine binding sites, could readily account for the very tight binding exhibited by receptors.

Finally we consider the possibility that the presence of a potential DNA-binding site, in addition to its thyroxine site, makes prealbumin a useful model of the thyroid hormone nuclear receptor. This possibility can be tested by verifying or contradicting the following predictions on the properties of the receptor that can be derived from the model: (1) it is a tetramer; (2) it is unusually stable and not easily dissociated into subunits; (3) its polypeptide chain is largely organised into β-structure; (4) it has two hormone binding sites; (5) it interacts with a segment of DNA 10–12 base pairs in length; (6) this segment contains a palindromic sequence of six to eight bases. Two other properties of the receptor, its molecular weight[18] and the linear arrangement of hydrophilic, hydrophobic and ionic groups in its hormone binding site[14], are not included in these predictions because they have already been determined, and are in agreement with the model.

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Separation of membrane currents using a *Paramecium* mutant

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The net membrane current of the electrically excitable membrane of Paramecium *during step depolarisations was measured using voltage-clamp techniques. The mutant, pawn B, lacks a functional Ca channel. Thus the difference between the total current measured in the wild type and the leakage and rectification currents measured in the pawn mutant is the Ca current. These findings were confirmed by ion substitution experiments. The possibility that inactivation may be apparent only, and caused by a hypothetical, superposed, Ca-induced, rectifying K⁺ current was eliminated.*

IN an attempt to study the molecular biology of excitable membranes in ways not possible in nerve, behavioural mutants with defective membrane functions have been isolated in *Paramecium*. *Paramecium* is a unicellular animal which swims by beating its cilia. The direction of its ciliary beat is controlled by the electrically excitable membrane[1,2]. Extensive electrophysiological studies produced evidence of the existence of Ca and K channels in the *Paramecium* membrane[3–8]. One of the difficulties of membrane-physiological investigations in general is that only the net transmembrane current or the voltage change due to this current is measured by the electrode. Analysis of the properties of

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a single kind of channel requires a separation of different ion currents. Traditionally, this is done with drugs or by ion substitution[10,11]. The same purpose can be accomplished by genetic means. We separate the inward Ca^{2+} current by comparing the total current in wild-type *Paramecium* and that in a mutant which lacks a functional Ca Channel[4,9]. To measure the total membrane current, we use constant voltage pulses during step depolarisations. We find that the Ca current inactivates in time, similar to the Na currents described in nerve[12] but different from Ca^{2+} currents described in barnacle muscle[13] and at the squid giant synapse[14]. This finding is supported by ion substitution experiments done on single wild-type cells. Suspicions are removed that the apparent time course of the Ca^{2+} current may be partially masked by a Ca-induced K^{+} rectifying current.

Wild type compared with the mutant 'pawn'

When wild-type paramecia hit a physical obstacle or enter an undesirable ionic environment, they avoid it by swimming backwards for a short distance and then swimming forwards again in a different direction[15]. The electrical excitability of the membrane controls backward swimming. A small depolarisation results in a graded action potential whose inward current is carried by Ca^{2+} (refs 3, 5). The increase in internal Ca^{2+} concentration then causes the cilia to reverse the direction of their power stroke (ciliary reversal) by a yet unknown mechanism[16].

Named after the chess piece, 'pawns' are mutants which cannot swim backward[17,18]. Electrophysiological studies traced the defect of these mutants to the malfunction of the Ca channels[4,9].

Paramecium tetraurelia, previously known as *P. aurelia*, species 4 (ref. 19) was used in these experiments. The wild type was stock 51S and the pawn mutant was stock d4-95, genotype *pwB pwB* (refs 4, 17). Voltage was clamped using standard techniques[20]. Cells, in a buffer solution containing 1 mM CaCl$_2$, 1 mM HEPES (Sigma), 0.5 mM KOH, 3.5 mM KCl, 10^{-7} M EDTA, *p*H 7.0, were impaled with microelectrodes filled with 3 M potassium acetate and having a resistance of 10–15 MΩ The potential was held at resting level and stimulus pulses were given at a frequency of about 1 Hz. The voltage stabilised at the command level 500 to 1,000 μs after a step change in the command. At the end of an experiment the voltage-measuring electrode was withdrawn to measure the reference potential. Activation of the Ca^{2+} inward current is fast, so that the beginning of activation could not clearly be seen, even in the best preparations. It is technically difficult to get stable preparations of *P. tetraurelia* with the low resistance electrodes which are required if the potential is to be changed quickly enough to see inward currents clearly.

Sample traces are shown in Fig. 1. The current traces from wild-type cells (Fig 1*a*, *c*) show a transient inward current lasting about 5 ms and then a steady-state outward current in response to a step depolarisation. A step depolarisation of pawn cells results in a steady-state outward current but there is no detectable inward current (Fig. 1*b*) even when viewed at high gain (Fig. 1*d*). Steady-state outward currents of pawn and wild type are not detectably different over the range of voltages studied (Fig. 2), and the voltage dependence of both peak and steady-state currents is similar to that in *P. caudatum*[21]. Depolarisations to nearly 0 mV from the resting potential of ~ -23 mV result in a transient inward current and a steady-state outward current whose magnitude is about equal to that of the leakage current. Steady-state outward currents increase steeply as a function of voltage for step depolarisation above 0 mV. As in other excitable membranes, this voltage-dependent outward current is called the delayed-rectification current, and it is probably due to the opening of K channels. At large depolarisations the outward current activates so rapidly that the inward current cannot be seen clearly.

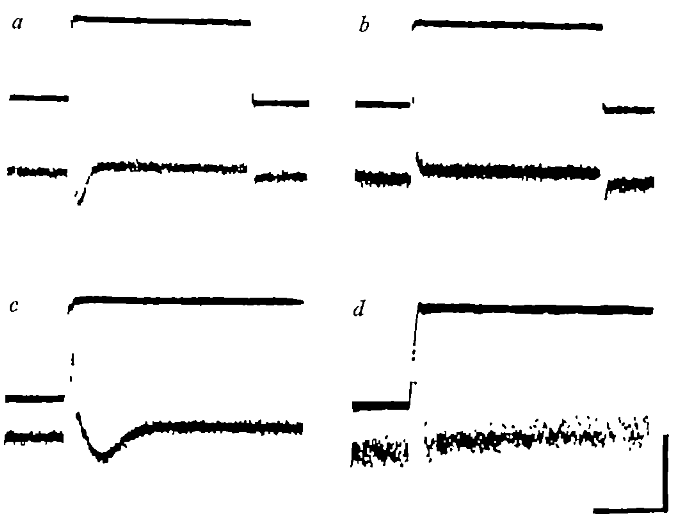
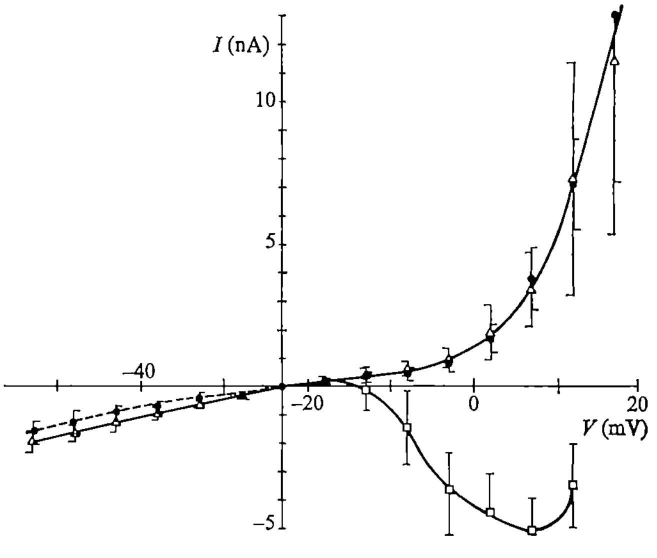


Fig. 1 Sample traces of voltage-clamp experiments in two strains of *Paramecium tetraurelia*. *a*, *c*, Records from wild type; *b*, *d*, records from the mutant 'pawn' cells. In each case the upper trace shows the voltage and the lower trace shows the associated current. In (*a*), a transient inward current is followed by a steady-state outward current. Record (*c*), from a different cell, shows a similar event at greater sweep speed. Record (*b*), and (*d*) show that pawns lack the transient inward current; (*d*) is recorded at higher gain and faster sweep than (*b*). Calibration: *a* and *b*, 20 mV, 10 nA, 10 ms, seven superimposed sweeps; *c*, 20 mV, 10 nA, 4 ms, 10 superimposed sweeps; *d*, 20 mV, 5 nA, 4 ms, 10 superimposed sweeps. Holding potentials are 20 mV, 24 mV, ? mV, and 25 mV for *a*, *b*, *c*, and *d*, respectively.

Pawn lacks a transient inward current, but there is no measurable difference in the characteristics of the steady outward currents between pawn and wild-type cells. The time courses of the transient inward current alone can be calculated by subtracting leakage and rectification currents as measured in pawn from the total current measured in wild type. Figure 3 shows one such calculation for cases with minimal rectification.

Fig. 2 Voltage–current relationship of peak inward (□) and steadystate outward (△) currents of wild type, and steady-state outward (●) current of pawn mutant. Steady-state outward currents were measured 25 ms after the beginning of depolarising pulses. Minimum inward currents resulted from hyperpolarising pulses. Data were taken from four wild-type cells with holding potentials 23±5 mV (mean ± s.d) and five pawn cells with holding potentials 23±3 mV. Points and bars represent mean current magnitudes ± s.d. Where the slope of the curve is greater, standard deviations are larger. There is no significant difference in the steady-state outward currents between the two stains.



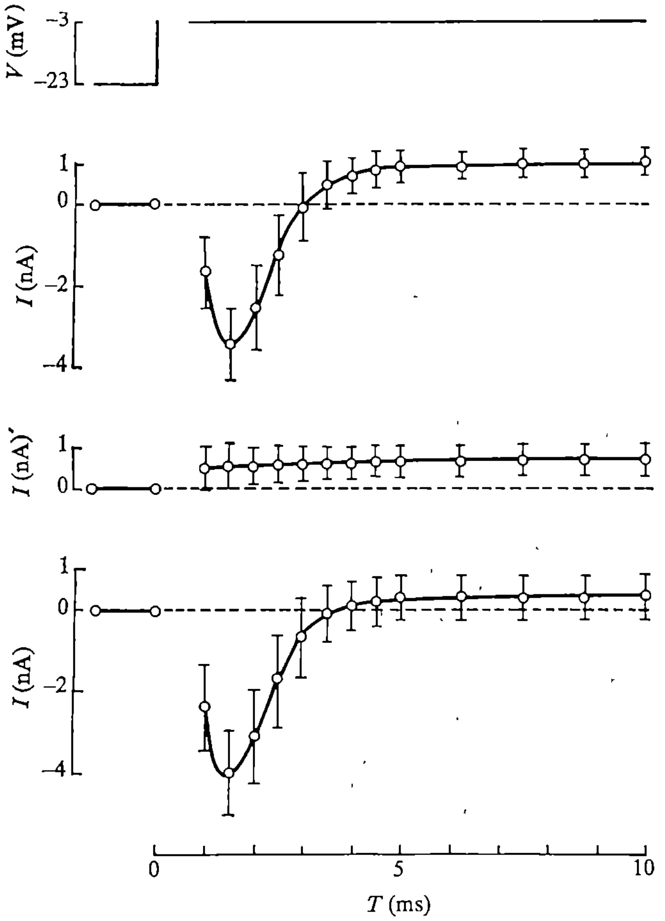


Fig. 3 Average time course of currents associated with step depolarisations of 20 mV in wild type (*a*) and pawn (*b*). Mean current magnitudes ± s.d were measured at various times from the same four wild-type cells and five pawn cells whose voltage–current characteristics are shown in Fig. 2 *c* shows the difference between current measured in wild type (*a*) and pawn (*b*) ± s.d. of the difference of the means. Measurements were not made in the first 1.0 ms after the step depolarisation because the voltage was not well controlled during this time.

Variation in measurements makes the comparison of single cells unreliable, so average time courses of membrane currents were determined from four wild-type cells (Fig. 3*a*) and five pawn cells (Fig. 3*b*) after a 20-mV step depolarisation. The difference of the two time courses (Fig. 3*c*) shows the time course of the Ca^{2+} current. The inward current in these experiments begins before the voltage has stabilised. Therefore, the early kinetics of activation are obscured. The Ca^{2+} current apparently inactivates.

Inactivation of Ca current

Figures 1 and 3 show that the inward current diminishes in time. Other experiments were done to test whether the Ca^{2+} current inactivates. To obtain a more accurate measure of the Ca^{2+} current from a single cell, a series of experiments was done in which 99% of the $CaCl_2$ in the external solution was replaced by $MgCl_2$. The results of such an experiment are shown in Fig. 4. The records obtained when the cell was bathed in buffer containing 0.01 mM $CaCl_2$ and 0.99 mM $MgCl_2$ resemble those of pawn. They lack the transient inward current but steady-state, outward currents remain unaffected. These results confirm our conclusion that the inward current is a Ca^{2+} current and that this current inactivates.

The effects of Ca^{2+} and Mg^{2+} concentrations on the properties of the membrane were studied systematically. Decrease of external Ca^{2+} lowered the membrane resistance. Increase of external Ca^{2+} increased the resistance and raised the measured activitation potential for the inward current. The measured activation potential for the inward current was also raised when Mg^{2+} was added to the standard 1 mM $CaCl_2$ buffer. These results suggest that surface-potential effects must be considered in studies of *Paramecium*, and that lowering the divalent cation concentration indirectly affects membrane resistance and the channel's apparent activation potential in addition to changing the driving force of Ca^{2+} across the membrane[22].

Acidic phospholipids in the paramecium membrane give the membrane a negative surface charge. This must cause a relatively higher concentration of cations near the membrane than in the bath. Changes in the ion composition of the bath, particularly in its divalent cation concentration, will alter the surface potential and ion concentrations in the vicinity of the membrane, and thus the electric field to which the membrane components are subjected may be quite different from that measured at a distance[23]. The experimental result that neither the measured activation potential for delayed rectification nor the hyperpolarisation currents are affected if Mg^{2+} is substituted for Ca^{2+} suggests that Mg^{2+} does substitute for Ca^{2+} in its effects on membrane resistance and surface potential but that it does not cross the membrane through the Ca channels[23,24].

The rate at which inactivation is removed when the membrane was held at resting potential was studied by stimulations paired at various intervals. A sample trace of such an experiment is shown in Fig. 5. Two depolarising pulses were given, each 5 ms long. An inward current is the response to a first depolarisation but it is lacking after the second. The time course showing the recovery of the inward current is shown in Fig. 5. It takes 80–90 ms for inactivation to be removed after a 5 ms pulse.

Ca-activated K conductance not involved

A Ca-acitvated potassium conductance has been shown to exist in a number of systems[25,26] that possess an inward Ca^{2+} current. It therefore seems possible that the observed loss in time of inward current is not caused by inactivation but by an activation by Ca^{2+} of an outward current which eventually cancels the inward current. The experiment shown in Fig. 6 demonstrates that this is not the case. If we

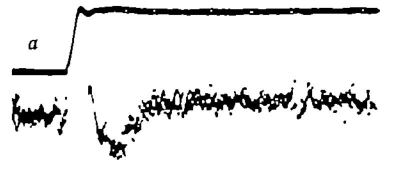


Fig. 4 Separation of currents in a wild-type cell by ion substitution *a*, Current response (noisy trace) to a step depolarisation (smooth trace) in a bath containing 1 mM Ca^{2+}. *b* Current, response to a similar depolarisation in the same wild-type cell when the bath was changed to one with 0 01 mM Ca^{2+} and 0.99 mM Mg^{2+}. The transient inward current seen in the upper trace is not seen when the external Ca^{2+} concentration is reduced but steady-state currents are equal This effect is reversible. Due to use of microelectrodes with relatively high resistances, unavoidable when voltage-clamping *P. tetraurelia*, a current oscillation follows the capacitative current at the beginning of the step depolarisation The membrane potential first was held at the resting potential, −25 mV, then depolarised to − 10mV in each case. Calibration bars· 20 mV, 10 nA and 4 ms. Each record shows four superimposed traces.

suppose that in the steady state there is a Ca-activated K^+ current component which cancels some of the inward current, then a sudden change of potential to more negative than E_K (ref. 21) ($E_K = -38$ mV in *P. tetraurelia*, unpublished observation) would reverse the direction of the K^+ current while the Ca^{2+} current should remain inward. Thus, the Ca^{2+} current and the hypothetical K^+ current which are supposed to cancel at -10 mV should add at a membrane potential below -38 mV. But, the observed hyperpolarising current is the same, with depolarising prepulse and without. There is, therefore, no measurable, long-lasting, Ca-activated K^+ current in the steady state which could be considered responsible for cancelling of an inward current. Ca-activated K channels may well exist in the *Paramecium* membrane (Y. Satow and C. Kung, in preparation). But, these channels seem not to be activated at internal Ca^{2+} concentrations or with kinetics relevant to excitation, in the conditions described in this paper. The relatively slow increase of the inward current during hyperpolarisation is due to activation of a different K conductance which causes an anomalous rectification (Oertel *et al.*, unpublished and ref. 27).

Concentration and conductance of Ca^{2+} per cilium

Two independent studies have shown that the excitation-related Ca channels are located mostly, and perhaps exclusively, in the cilia[28,29]. Thus it is likely that it is the local concentration of Ca^{2+} in the cilia, and not the concentration in the general cytoplasm, which controls beat direction. An order-of-magnitude estimate can be made of the increase in Ca^{2+} concentration in a cilium during an action potential. For this rough estimate we make the simplifying assumption that the time course of the Ca^{2+} current during an action potential is similar to that measured in voltage clamp conditions. Such an assumption seems reasonable, because the action potentials last so long that almost all channels should be open and inactivated[3,4]. 8×10^{-17} equivalents of Ca^{2+} are transferred during a 20 mV depolarising pulse. Taking the collective volume of all cilia of one cell to be 1.7×10^{-12} l, the change in Ca concentration in cilia would be about 2.3×10^{-5} M.

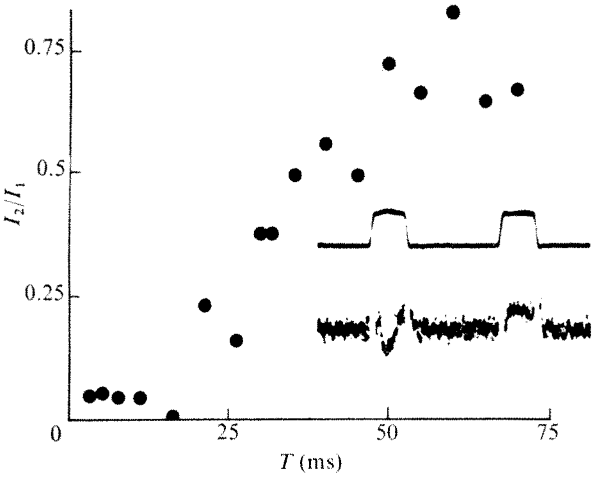


Fig. 5 Time course of return to resting state after inactivation in wild-type paramecium. Two 5-ms depolarisations each of 15 mV were given at various intervals. The ratio of the magnitudes of the second inward current to the first (leakage current subtracted) plotted as a function of time between end of first and second voltage pulse. Inactivation, nearly complete for about 20 ms, was removed after about 80–90 ms. Cell held at -18 mV between pulses. Sample trace is from a different cell with lower resting potential, but shows results more clearly because of smaller current swings after voltage changes.

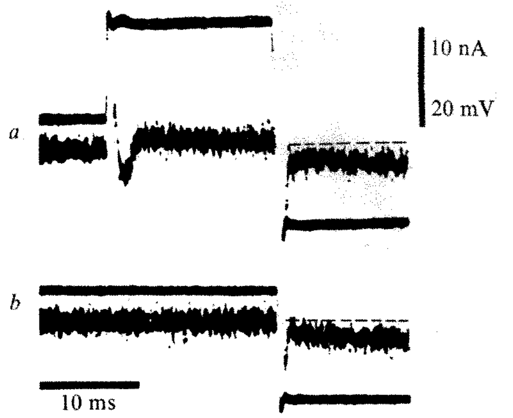


Fig. 6 Transience of inward Ca^{2+} current is not due to slow increase of a masking rectifying current but to an actual decrease of Ca^{2+} current (inactivation). *a*, Current response (noisy trace) to a step depolarisation followed by a hyperpolarisation (smooth trace). *b*, Current response of hyperpolarisation only without a depolarising prepulse. To detect hypothetical currents, assumed to cancel during depolarisation, current responses to hyperpolarisation are compared in *a* and *b*. If transcience of the inward current, seen when the cell is depolarised, is due to Ca^{2+} inactivation, the steadystate current should comprise only small rectifying and leakage currents. Hyperpolarisation to a potential (-48 mV) more negative than E_K (-38 mV) must reverse any postulated rectifying currents making all ion currents inward and additive. The observed current following depolarisation in *a*, however, is not detectably larger than in (*b*). Therefore, the inactivation, lasting for 80–90 ms, cannot be explained by postulated long-lasting rectifying currents.

Using paramecia whose membranes were made leaky with a detergent, Naitoh and Kaneko[16] showed that 10^{-6} M Ca is the threshold for ciliary reversal. Our estimate indicates that each action potential can deliver enough Ca^{2+} to cause ciliary reversal. Eckert[30] arrived at a similar conclusion by calculating how much Ca^{2+} was needed to charge the membrane capacitance.

It is also possible to make a rough estimate of the Ca^{2+} conductance per cilium. The maximum Ca^{2+} current measured is 4 nA for a depolarisation to 0 mV. If one assumes that the equilibrium potential for Ca^{2+} is about 150 mV, the maximum conductance per cell is 27 nmho, a value in close agreement with that calculated by Schein *et al.*[9], and the maximum conductance per cilium is only 5 pS.

Discussion

Indirect evidence further supports our finding that Ca channels in *Paramecium* inactivate. Naitoh and Eckert[3] and Naitoh, Eckert and Friedman[5] showed that the action potential of *Paramecium* is refractory. Since it also shows no undershoot which would indicate a maintained increased permeability to K^+, the refractoriness of the action potentials must be due to Ca inactivation. Eckert, Naitoh and Machemer[2] suggest that the *Paramecium* Ca current is at least partly transient. They found that small depolarising voltage-clamp pulses of several seconds produce only transitory reversals and that large and long depolarising pulses produce ciliary reversals which decrease in frequency with time. The transitory reversals observed by these authors last much longer than our calculated Ca^{2+} current. It is, however, difficult to compare time courses of currents and with those of ciliary reversal because little is known about how internal Ca concentration is regulated. Ultimately, it is internal Ca^{2+} concentration, not Ca current, which regulates reversal[2,16,31].

The Ca^{2+} action potential in *Paramecium* resembles Ca^{2+} spikes in metazoan systems[13,14,32–34]. In all cases, the action potentials are graded; Ba^{2+} and TEA^+, applied externally, change the action potential from graded to all-or-none[32,33].

But it is not possible to make generalisations about inactivation. Hagiwara[32] and Keynes *et al.*[13], for instance, report that the Ca^{2+} current in barnacle muscle does not inactivate, whereas Mounier and Vassort[32] show that the Ca^{2+} current in crab muscle does inactivate.

We have shown that the *pwB* mutation of *Paramecium tetraurelia* eliminates the transient inward Ca^{2+} current that occurs in the wild type on depolarisation. This observation enables us to separate the Ca^{2+} inward current, much as one uses tetrodotoxin to separate the Na^+ current in nerve cells, from the leakage and rectifying currents.

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letters to nature

Airborne studies of thunderstorm electrification

THERE is considerable controversy[1,2] surrounding the problem of thunderstorm electrification. Many scientists have maintained that precipitation acquires substantial charges and that field growth to breakdown magnitudes is a consequence of the separation under gravity of the precipitation and the oppositely charged cloud particles. Others have argued that precipitation is incidental to field growth, and have reported lightning from clouds in which the precipitation rates are very low. Of the charging mechanisms involving precipitation, the inductive mechanism[3,4] has been generally favoured. Under this mechanism rebounding collisions of small cloud particles with the underside of precipitation elements result in the removal from the latter of polarisation charge, and the maximum charge Q_m (pC) that can be acquired by an element of diameter d (mm) in a field E (kV cm^{-1}) is given by

$$Q_m = 5.5Ed^2 \qquad (1)$$

We summarise here the results of some airborne experiments conducted in the summer of 1976 in Florida, as part of the Thunderstorm Research International Programme, and subsequently in Socorro, New Mexico. The major objectives of this study were to determine whether substantial currents are carried on precipitation in thunderclouds, and to investigate the relationship between Q, E and d for precipitation particles—and thereby to test the applicability of equation (1) to thunderstorm electrification.

The measuring equipment was carried on the ONR/NMIMT Schweitzer aircraft. This was originally a drone, but it has been modified to accommodate a pilot. Its particular virtue—which renders it excellent for these experiments—is that it flies very slowly (around 40 m s^{-1}). Thus it has a minimal effect on any clouds through which it passes. Its ceiling altitude is 13 km.

The electric field in three mutually perpendicular directions was measured using five appropriately sited field mills. Simultaneous measurements of the charge and size of precipitation particles were made with a novel device mounted under one wing of the aircraft. An aluminium cylinder of length 80 mm and diameter 50 mm was mounted with its axis parallel to the airflow. The charge Q carried on a hydrometeor which passed through the device without touching it was sensed by induction and measured using a charge amplifier. A major problem with such a method is that spurious signals may arise from collisions of hydrometeors with the sensing cylinder. This was circumvented by making the time constant for decay of charge (resulting from such a collision) about 10 times longer than the transit time (and thus the duration of the charge-pulse) associated with the clean passage of a hydrometeor through the cylinder. The two types of pulse could be distinguished unequivocally and only the true ones (clean passages) were accepted. Various practical tests demonstrated that the sensitivity of the charge amplifier was consistent with that derived from an analysis of the circuit. The device was so arranged that it was impossible for hydrometeors to splash into the induction cylinder from any part of the aeroplane. Insulation loss in the charge amplifier—which happened occasionally in very heavy rain—was easily recognised from the recorded signals. Values of Q up to ±150 pC could be measured to an accuracy of ±5 pC.

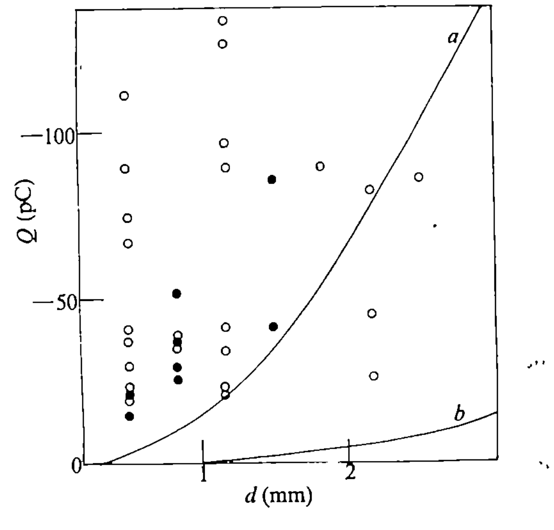
The coincident measurements of the size d of hydrometeors passing through the induction cylinder were effected by means of a shadowgraph technique. A beam of light passed across the cylinder, perpendicular to its axis, and half way along its length. It illuminated a linear array of 64 photo-diodes, spaced 100 μm apart, their line being perpendicular to the axis. When a particle passed cleanly through the beam illumination of some of the diodes was obscured and an electrical pulse was obtained whose magnitude was related in a known way to the size d of the particle. A flagging arrangement permitted the rejection of signals associated with particles which passed through the edge of the beam. A series of calibration experiments, employing stationary wires and also pellets of known size fired from a gun through the light beam illuminating the diode array showed that the values of d were accurate to ±0.3 mm. The range of values of d that could be measured was 0.5 to 4.4 mm. The device is uncomplicated, functioned consistently well and (by integration of the *d*-pulses) provided crude but sensible estimates of precipitation rates. The tape-recorded commentary of the pilot showed that his observations of the presence or absence of raindrops coincided accurately with the information given by the device.

There was no difficulty in identifying *Q*/*d* coincidences from our tape-recorded charge and size pulses. The spacing in time of coincident *Q*/*d* pulses was typically one or two orders of magnitude shorter than the mean intervals between successive *Q* or *d* pulses. The measurements of electric field, particle size and particle charge were telemetered to ground, as was the pilot's commentary (which was very important in these studies) and other measurements. In 1976 much of the effort was devoted to optimisation of the various instruments carried on the Schweitzer, and most of the measurements were devoted to horizontal penetrations near the bases of thunderclouds, where the vertical field was generally negative (that is, in opposition to the fine weather field).

The great majority of precipitation particles were found to carry negative charges. Over extensive horizontal distances, with vertical fields up to about -30 kV m^{-1}, the volume charge density on precipitation ρp—obtained by summation of the individual charge pulses—was in the region of -5 nC m^{-3}. The corresponding precipitation current j was around 15 nA m^{-2}. These values of ρp and j are of sufficient magnitude[5] and of the correct sign, to generate breakdown fields in the central regions of thunderclouds in the time generally considered to be available.

The number of coincident measurements of *d* and *Q* was limited because only about 20% of the particles entering the cylinder would pass through the light beam, and some insulation problems with the *Q* device were experienced in heavy rain. The coincidences obtained for two particular penetrations are displayed in Fig. 1. They show that no simple relationship exists between *Q* and *d*. Furthermore, the charges carried on the precipitation are usually far greater than the maximum (curve *a*) that they could acquire under an inductive mechanism operating even in regions where the field was at breakdown value. The charges on some of the smaller particles were close to the Rayleigh bursting limit and many of these hydrometeors carried charges in excess of those which would be required for levitation in positive fields of breakdown magnitudes.

Fig. 1 Measured coincident values of charge *Q* and size *d* of precipitation elements during two penetrations on 9 August 1976. ○, Penetration 3; ●, penetration 4. Curve *a* from equation (1) with $E = 300$ kV m^{-1} Curve *b* from equation (1) with $E = 21$ kV m^{-1}, which was characteristic of the field values measured during these penetrations.



It was not possible to make precise estimates of precipitation rate p from the *d*-pulses, because the sampling volume was small. It was established, however, that in regions where the values of ρp and j were large p did not generally exceed about 10 mm h^{-1}; and on several occasions appreciable values of ρp were measured when p was below 1 mm h^{-1}. These observations of low precipitation rate were confirmed by the visual observations of the pilot.

Records from flights made on several days confirm our general findings—that precipitation currents and charge densities can be very high, even when precipitation rates are low, and that the individual values of *Q* are inexplicable in terms of the inductive theory. Thus it appears that precipitation may well play a major role in thunderstorm electrification, but that the mechanism responsible for the charge transfer remains to be identified.

A full discussion of these experiments will be described in a lengthy paper, currently in preparation.

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Pattern recognition applied to uranium prospecting

PATTERN recognition techniques provide one way of uniting quantitative and descriptive geologic data for mineral prospecting. A quantified decision process using computer-selected patterns of geologic data has the potential of selecting areas with undiscovered deposits of uranium or other minerals. When a natural resource is mined more rapidly than it is discovered, its continued production becomes increasingly difficult. For example, Lieberman[1] has noted that, although a considerable uranium reserve may remain in the U.S.A., the discovery rate for uranium is decreasing exponentially with cumulative exploration footage drilled. Pattern recognition methods of organising geologic information for prospecting may provide new predictive power as well as insight into the occurrence of uranium ore deposits. Often the task of prospecting consists of three stages of information processing: (1) collection of data on known ore deposits; (2) noting any regularities common to the known examples of an ore; (3) selection of new exploration targets based on the results of the second stage. Here we describe a logical pattern recognition algorithm that implements this geologic procedure to demonstrate the possibility of building a quantified uranium prospecting guide from diverse geologic data.

We chose to test prospecting by pattern recognition in an area with dimensions 85 × 90 km of the Colorado–Utah border between 108°30′W to 109°30′W and 38°0′N to 38°45′N. This area contains many commercial uranium deposits as well as much unproductive area[2]. Because this part of the Colorado Plateau is relatively well understood and explored, pattern recognition classification of barren and uranium-bearing areas may be verified by comparison with the results of past field exploration and development.

In the study area, uranium deposits occur chiefly as pene-concordant ore bodies in the Jurassic Morrison formation, a continental sandstone interbedded with mudstone. Most geologists agree with Hostetler and Garrels[3] that the deposits were formed when uranium minerals precipitated from groundwater flowing through gently dipping sandstone beds with locally strong reducing capacity. Fossil plants and humic material seem the most likely sources of reducing agents. Uranium may have been derived from distant upland rocks, basement rock, overlaying beds containing volcanic ash, updip leaching of the host formation, or hydrothermal circulation associated with intrusives.

The principal recognition algorithm used here has been described in full elsewhere[4,5]. In the present experiments, a computer searches geologic data for discriminants (called traits) that can be used to classify locations as either uranium-bearing or barren. In a training stage, the locations of known uranium deposits are presented to the computer together with geologic data at a number of sites in a study area. In the training district, the goal of data analysis is the identification of a set of geologic traits that discriminate producing areas from barren sites. This set of traits may then be taken to unexplored areas and used to predict the location of uranium-prone sites. Perfect classification, even of locations in a training district, is not expected because adequate geologic data may not be available, all ore bodies in a district may not have evolved similarly, some undiscovered ores may be incorrectly designated as barren, and instructions to the computer may not be optimal.

Many types of data, from standard geologic maps, geophysical, geochemical, and radiometric surveys and satellite imagery are now used in commercial exploration for uranium. In the present study, only readily available structural, stratigraphic, and geomorphic information was used in computerised prospecting. This information does not include many significant geologic features of uranium-bearing areas. Because major pre-ore structural features may have strongly influenced the flow of uranium-bearing groundwaters, however, this simple geomorphic information should have a considerable role in ore body location and will demonstrate the potential of pattern recognition techniques in prospecting.

Before analysing geologic data for patterns, several individual geologic features are tested to find some that are particularly characteristic of producing or of barren areas. The selection of features for recognition is based on estimates obtained from the training district of state-conditional probability density functions for the occurrence of features. Geologic data are simplified to binary-valued features, and a string of binary digits is used to describe each location (examples of the data available for this study are given in Table 1). The computer then uses this data base of binary features to contrast barren and producing areas by constructing more complex logical descriptions of these two classes of locations from the binary feature primitives. In this way, the computer may reveal logical relations particularly useful in uranium prospecting within large quantities of diverse geologic data.

Combinations of one, two and three binary values of features taken together constitutes traits. With N binary features

$$2N+4C_N^2+8C_N^3$$

traits are examined, where C_N^M is the number of combinations of N things taken M at a time. With the 19 features of Table 1, for example, no geologist could hope to examine all of the 8,474 possible traits. A computer is used to examine all these and to select the few traits most characteristic of barren and of producing areas. A trait characteristic of producing areas is selected if a trait occurs at more than K_1 producing locations and fewer than K_1* barren locations; similarly, K_2 and K_2* define characteristic traits of barren areas. In the present experiments, K_1, K_1*, K_2, and K_2* were about 60%, 10%, 40%, and 10% of producing and barren areas, respectively. In a final classification stage, if the number of producing traits exceeds the number of barren traits present at a point, the point is classified as uranium-bearing by the computer, otherwise it is classified as barren.

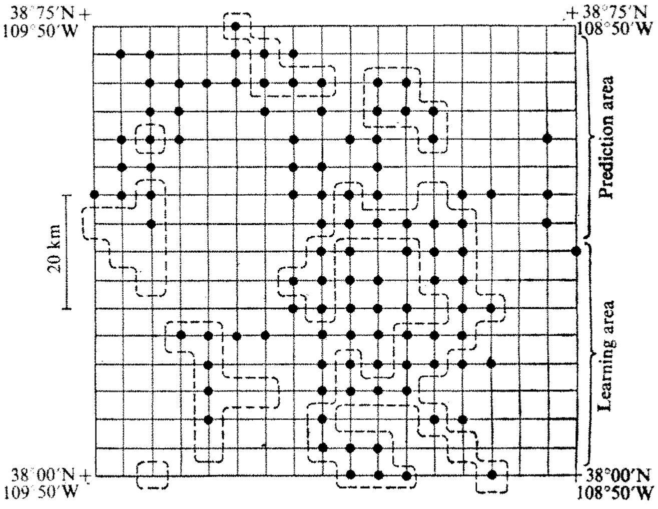
Table 1 Examples of features for recognition of uranium deposits

Position of location between nearest surrounding drainage divides
Lowest elevation within 3 km
Highest elevation within 3 km
Topographic gradient
Average elevation
Maximum bed dip within 5 km
Distance to Quaternary Sediment
Distance to major river
Distance to syncline axis
Distance to NE-trending basement fault
Distance to basement fault intersection
Distance to exposed crystalline rock
Number of formations outcrop within 2.5 km
Distance to anticline
Distance to Cretaceous intrusion
Is nearest anticline closer than nearest syncline?
Convergence or divergence of bed dip over Morrison formation
Does Morrison outcrop in 2.5 km?
Structure contour on Dakota sandstone base

We tested how well the computer can learn in one region and predict in another in a simulated exploration survey. The computer was given geologic data and the locations of known mines in the southern part of our grid. After surveying possible features and developing traits on 162 points in the southern part of our grid, geologic data for 144 points in the northern part of the grid were given to the computer with instructions to select the most likely uranium prospects based on results of learning in the south. Results of this recognition and prediction are given in Fig. 1.

In the training area, 80% of producing points and 82% of barren points were correctly recognised. Most misclassified barren points are adjacent to known deposits, so that these

Fig. 1 Results from Experiment 1. The computer was trained on the southern part of the grid and predicted in the north. Grid points classified uranium-bearing on the basis of traits are indicated by ●. Grid points with known uranium production are within the dashed contour (production data from Williams, 1964).



errors are not unreasonable. With a 5-km grid spacing, some errors are expected as adjacent grid points may have similar descriptions in our limited feature space.

In the north, despite the small training sample and the limited information available in features, the computer did pick as good prospects some points in each of five producing zones. Correct recognition rates in the north are comparable with those in the south, 83% for producing areas and 74% for barren areas. Since no set of locations will resemble the training set as much as the training set itself, an error rate in prediction comparable with that in learning may be regarded as excellent performance. Also, some of the errors in classifying barren points may represent undiscovered deposits.

To further test the method, training and prediction was carried out for the entire area of Fig. 1. This differs from the procedure of learning in one area and predicting in another, and suggests using pattern recognition to identify overlooked deposits within a mining district. Results comparable with those from our Experiment 1 were obtained, attesting to the stability of the method against large variations in the amount of learning material. We gain additional confidence in computerised trait selection because traits are always geologically reasonable, though it is not obvious before applying our procedure which features will be combined into traits for optimum recognition.

To demonstrate that the geologic patterns found by our procedures are not random accidents, control experiments to test for self-deception were performed. Although not a part of standard pattern recognition procedures, some such test is important when considering complex geologic systems. The computer was given spurious but realistic geologic information by holding geologic data at each grid point fixed but rotating the spatial classification pattern to be duplicated by 180°. This rotated pattern retains the spatial distribution and degree of order of the original classification. In this experiment, all 306 grid points were again used as a training set. No traits were found to satisfy the K/K^* conditions imposed on traits from real data. Relaxing the K/K^* conditions to obtain traits, the algorithm could force this artificial data barely above the 50% correct recognition rate expected from random binary selection of prospects. The failure of recognition based on spurious but spatially realistic information (as well as similar failures of recognition based on randomly generated binary features) lends confidence to results based on real data.

In these and related experiments, a contextual/decision theoretic pattern recognition procedure was able to extract significant logical relationships between diverse types of geologic data and to use these relationships in correct recognition of uranium-producing areas. Correct recognition rates up to 85% for uranium-producing areas with few false positives indicates the potential of pattern recognition in selecting exploration targets. When comprehensive data from standard geologic maps, LANDSAT imagery, geochemical, radiometric, and geophysical surveys are available, computerised prospecting procedures may provide a powerful new approach to predicting new areas of commercial resource potential. A complete description of this work will be published elsewhere.

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An important ground surface sink for atmospheric nitrous oxide

ONE of the more remarkable achievements of atmospheric chemistry is the identification by Crutzen[1], in 1970, of nitric oxide (NO) as the main natural agent for the destruction of ozone produced photochemically in the stratosphere. The principal source of stratospheric NO is the reaction of excited oxygen atoms (O^1D) with nitrous oxide (N_2O)[2], present as a normal constituent of the Earth's atmosphere. N_2O was first observed, in 1938, by Adel[3] in the absorption bands in the 7.8 μm region of the solar spectrum, but knowledge of its atmospheric life cycle is still very incomplete because of the few measurements available of its atmospheric concentration and limited information on atmospheric sources and sinks. The soil has been suggested as a major source of atmospheric N_2O[4] and the ocean has been proposed to act both as a source[5] and as a sink[6]. The only sink so far positively established by atmospheric measurement lies in the stratosphere, being predominantly photodissociative in nature with an additional contribution from the chemical reaction with excited oxygen atoms[4,7]. We report here evidence for the existence of an appreciable, but unidentified, ground surface sink mechanism.

Interest in monitoring atmospheric N_2O has been stimulated recently, both from realisation of its important role in ozone chemistry, and concern that perturbation of the ozone level in the stratosphere may occur due to enhanced N_2O emissions from the soil arising from increasing world-wide application of nitrogenous fertilisers. McElroy[6] has predicted that a 20% reduction in the concentration of stratospheric ozone may occur by the first quarter of the twenty-first century if present trends in the use of nitrogenous fertiliser continue.

The recent development[8] of the heated electron capture detector for gas chromatographic determination of N_2O has facilitated the measurement of ambient levels of N_2O in air without the need for previous concentration of the air sample[9,10].

In the following, we describe some measurements of N_2O made using such a detector at AERE Harwell in July and August 1976. Outside air was sampled at 5 m above ground level and drawn through a 6 feet stainless steel tube into a 5 cm^3 sample loop. The air in this loop was injected every 2 h into a PYE 104 gas chromatograph equipped with an electron capture detector operating at 350 °C. A 9 feet Porapak QS column, maintained at 27 °C, was used to separate N_2O using a 90% Ar–10% CH_4 mixture as carrier gas flowing at 30 $cm^3\ min^{-1}$. The apparatus was operated continuously for a five-week period commencing 7 July and ending on 9 August; our data was obtained in a period of extremely stable operation from 24 July to 8 August. This was demonstrated both by negligible changes in observed standing current of the detector ($1.00 \pm 0.03 \times 10^{-9}$ A) and by minimal changes in the signal displayed by injecting a standard sample at regular intervals. (0.278 ± 0.005 p.p.m.). During the same period outside air samples gave values ranging from 0.233 to 0.315 p.p.m.

The most important aspect of the data, shown in Fig. 1, is the observation of a regular diurnal variation of N_2O concentration on each of the 11 days shown. A minimum was nearly always recorded in the early hours of the morning and a maximum was recorded in the late afternoon or evening. Since the amplitude of this diurnal variation was only about 10% of the mean value, we have taken particular care to establish that it was not due to diurnal changes in instrument sensitivity associated either with changes in instrument conditions such as ambient temperature, or with changes in the concentrations of other atmospheric species such as water vapour or carbon dioxide. The observations, therefore, seem to indicate a real physical phenomenon which can only be explained either by some process which increases the N_2O concentration during the day, or alternatively depletes the N_2O concentration at night. Pierotti and Rasmussen[11] have noted that the N_2O concentration

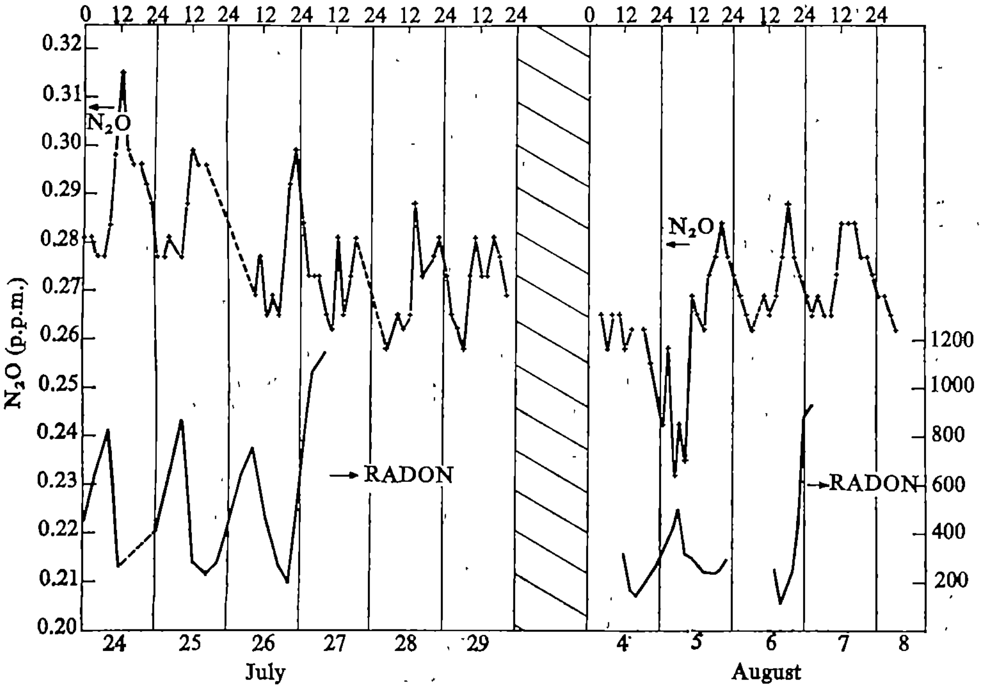


Fig. 1 Daily variation of N_2O and radon from 24 July to 29 July 1976, and from 4 August to 8 August 1976 (– – –, data pattern uncertain).

in power plant plumes is increased above the background level, but there is no reason why such combustion processes should lead to regular increases of the type shown in Fig. 1.

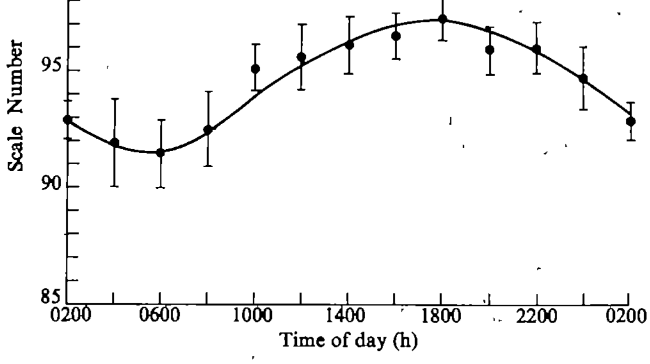
It seems rather that N_2O exhibited diurnal behaviour typical of gases such as sulphur dioxide, ozone and peroxyacetyl nitrate (PAN) which are absorbed at ground level surfaces[12,13]. Measurements of such gases made near the ground show a reduction in concentration at night, as they become depleted in the shallow layer of stable air which develops below the nocturnal inversion, and a rise during the day, as solar radiation gradually breaks up the inversion and allows fresh material to mix downwards. Inversions developed on many nights during the observational period which was characterised by anticyclonic weather conditions and clear skies.

Such diurnal behaviour is anti-correlated with that of radon[14] which is emitted rather than absorbed, at the ground surface. Included in Fig. 1 are the atmospheric radon concentrations, also measured at Harwell with continuous monitoring equipment, which showed this strong anti-correlation with the N_2O observations on several days during the period. A possible alternative explanation would be a gas-phase reaction with another gas released at ground level, but this is unlikely with a gas as unreactive as N_2O and no suitable candidate reactions have been suggested in the literature. Thus we conclude that absorption of nitrous oxide, probably by vegetation or the soil, is the most likely explanation of our observations though the mechanism by which this occurs is not clear.

Matsubara and Mori[15] suggested that N_2O would be removed by soil microorganisms and some recent investigations by Blackmer and Bremner[16] provides experimental evidence that soils can possess more potential to act as a sink than as a source of N_2O in conditions favourable to denitrification. These observations indicate that a sink other than destruction in the stratosphere may be significant for atmospheric N_2O. If the deposition is occurring irreversibly then the size of this sink can be estimated from the deposition velocity (v_g), which is related to the exponential decay constant λ, governing the rate of concentration decrease by the simple expression $\lambda = v_g/h$ where h is the height of the stable layer from which deposition is occuring[12,17].

Figure 2 illustrates the average diurnal pattern for the period in question. This was derived by assigning a value of 100 to the maximum N_2O concentration observed during any 0000–2400 cycle and scaling the other measurements accordingly. For the days considered, the scale numbers were then averaged for each respective sampling time. The average value of λ for decay of N_2O between 1800 h and 0600 h can be derived from Fig. 2, using a value of 0.280 p.p.m. for the mean N_2O concentration during the period. The magnitude of h is unknown, but, since it is the same for all gases undergoing deposition, the v_g for N_2O can be obtained directly by comparison with data on the average diurnal variation of ozone at Harwell over the same period. The value of λ for N_2O from Fig. 2 is $(5 \pm 0.7) \times 10^{-3}$ h^{-1} and the corresponding λ for ozone is (0.12 ± 0.02) h^{-1}. Since the average deposition velocity for ozone observed in similar conditions is about 0.3 cm s^{-1} [18] we calculate a value for N_2O of 1.25×10^{-2} cm s^{-1}. If, in the absence of further information, we assume this value is typical of all land surfaces then we can estimate the magnitude of the global sink for N_2O. The mean atmospheric lifetime of a species being removed by deposition at the Earth's surface is equal to H/v_g, where H is the equivalent height of the atmosphere (8.3 km) and v_g is the mean deposition velocity for the earth's surface. Assuming that deposition occurs all year at the same rate but only on land surfaces (0.26 of the total surface) then the life time of N_2O due to this sink is 8.1 yr. Taking a mean mixing ratio for N_2O of 0.3 p.p.m.[19],

Fig. 2 Average diurnal variation for N_2O. ⊕, Average scaled number value for corresponding injection time; I, ±1 standard error of the mean.



the magnitude of this sink is estimated at 320 Mt yr^{-1}. This figure, which can only be very approximate at this stage, is twice the surrent estimated combined ocean source, 135 Mt yr^{-1} [5], and soil source, 25 Mt yr^{-1} [4]. It is more than an order of magnitude greater than the figure of 13 Mt yr^{-1} for the stratospheric sink[20]. Use of the mean lifetime figure of 8.1 yr would substantially reduce McElroy's estimate of the reduction in stratospheric ozone due to the use of nitrogenous fertilisers.

Further data in a variety of conditions are needed before the global magnitude of this sink mechanism, which seems larger than any other, can be estimated with confidence.

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Generation of a supported iridium catalyst of extremely high dispersion

THE preparation of supported metallic catalysts in a very high state of dispersion—that is, with a very small average metallic particle size—is of considerable current interest. Not only is the surface area per unit mass of metal thus increased, but if the metallic particles are sufficiently small, their intrinsic catalytic properties may be different from those of the massive metal. On the whole, changes of the latter sort seem to be found when the particle diameter is less than about 2 nm (refs 1, 2). The use of a metallic cluster carbonyl (MCC) as a means of preparing a dispersed metallic catalyst has been the subject of preliminary exploration[3,4,5]. Although it was indicated that this route could yield highly dispersed metal, it was clear[3] that considerable cluster aggregation occurred during thermal decarbonylation and hydrogen reduction (at 573 K). This note reports the preparation from $Ir_4(CO)_{12}$ of a supported iridium catalyst in a very high state of dispersion.

The preparative details are: the support (Wohlm γ-alumina, 200 m^2 g^{-1}) vacuum-dried at 380 K for 15 h, was impregnated using the method of incipient wetness with a solution of $Ir_4(CO)_{12}$ used as obtained from Alpha Chemical Inc in cyclohexane (0.4 mg cm^{-3}, AR grade saturated). After standing for a few hours, the solvent was removed under vacuum at room temperature, and the sample was then heated to 623 K under vacuum for 4 h. The sample was then heated in hydrogen at 623 K for 15 h, and was then evacuated to better than 1 mPa at the same temperature. At this point, the catalyst was ready for characterisation by gas adsorption (H_2 or CO) or by electron microscopy. Catalyst compositions were determined by X-ray fluorescence.

The results for catalyst samples with loadings of 0.31 and 2.48 wt % iridium (dry weight basis) are given in Table 1. The extent of gas uptake over the pressure range 0.3–3 kPa was virtually independent of pressure and is taken to correspond to monolayer adsorption. In the case of iridium it is reasonable to take the monolayer chemisorption stoichiometry for hydrogen, $X_m(H_2) = 2$ (1), that is each surface iridium atom carries one hydrogen atom at monolayer coverage. On this basis the average iridium particle diameters were computed which are also included in Table 1 for comparison with the electron miscroscopic data.

Table 1 Characterisation of supported iridium catalysts

	Gas adsorption, ‡ 293 K 0.3–3 kPa (10^{21} molecules per g Ir)		$N_{(s)H}/N_{(T)Ir}$†	$N_{(s)CO}/N_{(T)Ir}$†
	H_2	CO		
$Ir/\gamma\text{-}A1_2O_3$ 2.48 wt % Ir	0.67	1.45	0.43 (2.5 nm)*	0.46
$Ir/\gamma\text{-}A1_2O_3$ 0.31 wt % Ir	1.51	7.66	1.0 (<1.1 nm)*	2.44

* Average iridium particle diameter obtained from $N_{(s)H}/N_{(T)Ir}$ and $X_m(H_2) = 2$.

† Obtained using the result that 1 g Ir contains 3.13×10^{21} atoms: $N_{(s)}$ and $N_{(T)}$ number of atoms at surface and in total respectively.

‡ Corrected for adsorption on the support.

Two main conclusions emerge from Table 1. For the catalyst for which $N_{(s)H}/N_{(T)Ir}$ (which equals the metallic dispersion $D = N_{(s)Ir}/N_{(T)Ir}$ by virtue of the value assigned to $X_m(H_2)$) is less than unity, there is reasonable agreement between $N_{(s)H}/N_{(T)Ir}$ and $N_{(s)CO}/N_{(T)Ir}$ at monolayer coverage, indicating $X_m(CO) \simeq 1$. However, in the case of the catalyst with the lower iridium loading of 0.31 wt % Ir for which $N_{(s)H}/N_{(T)Ir} = 1$, the value of $N_{(s)CO}/N_{(T)Ir}$ indicates $X_m(CO) = 2.44$. Although it is known that for extremely highly dispersed platinum the monolayer chemisorption stoichiometry ($X_m(H_2)$) may fall somewhat below two[2], we have not observed similar behaviour with extremely highly dispersed iridium. Bearing in mind that $D = 1.0$ is consistent with a range of particle sizes below a limit of about 1.1 nm in the case of iridium, and also noting that for this catalyst we failed to resolve any metallic particles electron microscopically, it is clear that the average iridium particle size was extremely small, certainly <1 nm. Since one would expect a range of particle sizes, it is reasonable to expect that some proportion of the iridium was retained as Ir_4 clusters. It remains to be assessed whether $N_{(s)CO}/N_{(T)Ir} = 2.44$ indicates that this proportion was substantial. Comparison with some results of Della Betta[6] for highly dispersed ruthenium catalysts suggest that the answer is probably in the negative. Della Betta studied the adsorption of H_2 and CO for average Ru particle sizes in the range 1.1–2.5 nm (evaluated from H_2 chemisorption data, and assuming $X_m(H_2) = 2$); the corresponding values for $N_{(s)CO}/N_{(T)Ru}$ were in the range 2.3–3.8. Clearly, with a metal for which a molecular cluster carbonyl exists ($Ru_3(CO)_{12}$ in the case of Ru), $N_{(s)CO}/N_{(T)M}$ can approach a value characteristic of the MCC even though the metallic particle size may be rather larger than that existing in the MCC itself.

Comparison with the results of Smith *et al.*[4] suggests that unaggregated metallic cluster skeletal units generated from metallic cluster carbonyls, cannot be retained on these supports if the temperature rises substantially above room temperature. This is probably a consequence of the facile migration of these units across these support surfaces at elevated temperatures. It may prove possible to overcome this problem by designing supports with surface sites of high binding energy at which the metallic cluster skeletal units may be immobilised, or to restrict skeletal unit migration by controlled pore structure of the support. These approaches are currently under investigation.

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Hydrogen in the Earth's core

THE density of the Earth's core is 8–10% less than that of pure iron at the same pressure and temperature[1] and this is usually interpreted as implying the existence of a substantial amount of light element(s) in the core. Many possibilities have been considered[1], with sulphur and oxygen[2] receiving most recent attention, but in this note the discussion is limited to hydrogen's contribution to the density deficit. It is shown that hydrogen is not only effective in decreasing the density but is also highly soluble in iron in the relevant conditions. Only about 1% by mass of hydrogen is required in the Earth's core to explain the density deficit, and this corresponds to a hydrogen–iron mass ratio that is only about one-tenth of that present in type I carbonaceous chondrites[3]. The significance of hydrogen as a contributor to the density deficit is, therefore, determined by the extent to which the Earth-forming matter consisted of low temperature condensates.

Hydrogen was prominent in the earliest discussions of core composition[4,5] but since it enters iron as interstitial atoms, Ringwood[6] proposed that it could not significantly contribute to the density deficit. In fact, the interstitial hydrogen substantially expands the host metal, and the zero pressure hydrides of transition metals are typically 10–20% less dense than the pure metal[7], in contrast to ionic or covalent hydrides. This has frequently been interpreted as the formation of the large (but highly compressible) H^- ion[7], but the thermodynamics are better understood in terms of a screened proton model[8], according to which the hydride can be thought of as an alloy of the transition metal and metallic hydrogen.

For simplicity, consider a host metal characterised by rigid ion cores of size r_c and effective charge Z (not necessarily an integer) immersed in an essentially uniform compensating electron gas. The energy per ion of the alloy $M_{1-x}H_x$, where M is the host metal, is then

$$E = (1-x)E_M(V_M)+xE_H(V_H)+\delta E_{MH} \qquad (1)$$

where V_M is the volume occupied by Z conduction electrons, $V_H = V_M/Z$, E_M and E_H are the energies per ion of the pure host metal and pure metallic hydrogen[9] respectively, and δE_{MH} is a small correction term (which includes the M–H interaction). Neglecting δE_{MH}, the small x limit gives

$$x = \frac{1}{\rho}\left(\frac{\partial \rho}{\partial x}\right)_\rho \simeq \frac{P-P_H-B}{ZB} \qquad (2)$$

where ρ is the mass density, P is the pressure, B is the host metal bulk modulus, and P_H is the pressure that metallic hydrogen would have if it had the same conduction electron density as the host metal. At pressures less than a few megabars, $P_H < 0$ and $B \gg P$ or $|P_H|$, so $\alpha \simeq -1/Z$, and the model approximately predicts the observed density decrease.

According to the empty core pseudopotential model[10], the known equation of state for iron[11] is consistent with $Z \simeq 2$ near $P = 0$ and $Z \simeq 3$ at Mbar pressures, both with $r_c \simeq 0.49$ Å. More realistic models for iron yield similar results for α. Since $|\alpha|$ decreases as P increases, the hydride is more compressible than the host metal, but not to such an extent as to be incompatible with the known pressure–density relationship in the Earth's core. An alloy with composition similar to FeH would explain the observed core density. Inclusion of δE_{MH} in the calculation actually decreases the required amount of hydrogen slightly. Thomas–Fermi calculations[12] predict that a much larger amount of hydrogen is needed, essentially because that method overestimates the conduction electron density.

Just above the iron melting point, the hydrogen solubility[8] for $x \ll 1$ is $x \simeq 0.015\ P^{1/2}$, where P is in bars, so a pressure of a few kbar suffices to form an FeH composition. At much higher pressures, where the coexisting molecular hydrogen ceases to be an ideal gas, the hydride should form even at $T = 0$ K, because the enthalpy of molecular hydrogen increases more rapidly than that of metallic hydrogen[9]. In the low temperature limit

$$x \simeq \exp(-\Delta H/k_B T) \qquad (3)$$

where ΔH is the enthalpy of hydride formation per proton and T is the temperature. At $P = 0$, $\Delta H = \Delta H_0 \simeq 0.25$ eV (ref. 8). At $P \neq 0$, equation (1) with $\delta E_{MH} = 0$ implies

$$\Delta H \simeq \Delta H_0 + P\left(V_{el} - V_{H,mol} - \frac{P_H}{B}V_{el}\right) \qquad (4)$$

where V_{el} and $V_{H,mol}$ are the volume per conduction electron in the hydride and volume per proton in the molecular hydrogen phase, respectively. From the known properties of molecular and metallic hydrogen[9], $\Delta H < 0$ if $P > 200$ kbar. Allowances for $\delta E_{MH} \neq 0$ and finite temperature in the Earth's core do not significantly alter this prediction for hydride formation. At pressures much higher than that in the Earth's core, hydrogen may again become insoluble[13].

The proposed hydride would be an excellent conductor, perhaps even better than pure iron (since metallic hydrogen conducts electricity or heat over 10 times better than iron at the same pressure and temperature[14]). It would also have a lower melting point than pure iron, and other properties which are compatible with present knowledge of the Earth's core.

If at least 10% of the Earth-forming material was a low temperature condensate (containing hydrogen in the form of water) as many models require[1,2], then the proposed core composition is possible. The extent to which hydrogen (or water) was lost during the heating accompanying Earth formation depends on the detailed pressure and temperature history. If, for example, the water reacted with iron at a pressure such that most of the released hydrogen could enter in solution with iron, then volatile retention could be high. The decreasing abundance of water with increasing metamorphic grade in chondrites is not an argument against volatile retention, since the hydrogen is only retained at high pressure, and would be expelled if the pressure were subsequently released. In conclusion, hydrogen is conceivably the major cause of the density deficit in the Earth's core, but is most probably just one of several contributing causes.

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Geochronology of some blueschists from Ile de Groix, France

THE Ile de Groix series consists of metavolcanic and metasedimentary rocks, with or without glaucophane. These rocks belong to a South Brittany polymetamorphic complex which is named the 'domaine de l'Anticlinal de Cornouaille' by Cogné[1]. In this complex, anatectic granites occur in the midst of a migmatitic and gneissic terrain[1,2]. Some metasedimentary and metavolcanic rocks which occur in the terrain are cut by Hercynian granites. The Ile de Groix series[1,3,4] is characterised by high pressure mineral assemblages with glaucophane, jadeitic pyroxene and lawsonite[5]—these assemblages are unknown on the adjacent mainland. The series is considered to have formed during two metamorphic periods: the first produced high pressure–low temperature mineral assemblages and the second produced a recrystallisation of mineral assemblages in greenschist facies[6]. In this note, we shall present new isotopic age data on various rocks and minerals from Ile de Groix, hoping to delineate the time sequence of original magmatism, sedimentation and subsequent metamorphism.

Six phengitic mica samples were separated from metavolcanic rocks and three from possibly metatuffaceous sediments. They were analysed for Rb and Sr concentration and Sr isotopic compositions. The ages calculated from five whole rock–phengite pairs, two epidote–phengite pairs and two 0.705-phengite pairs are clustered in the range of 340 to 375 Myr (λ $^{87}\text{Rb} = 1.42\times10^{-11}\ \text{yr}^{-1}$) (Fig. 1). K/Ar measurements, by J. C. Hunziker, on four of these samples yield slightly lower ages (340 to 360 Myr), but are, nevertheless, within error of the Rb–Sr ages (Fig. 1); a paragonite gives 273 ± 14 Myr.

The mica ages could be interpreted as representing either the time of crystallisation or cooling[7], but the actual scatter of these age data suggests the effect of cooling. Thus the age of 375 ± 15 Myr is considered as a minimum for the crystallisation of phengitic mica and thus also for the time of high pressure metamorphism. The paragonite age is interpreted as the final cooling event.

This interpretation does not agree with Carpenter and Civetta[8]. They obtained K–Ar ages on three glaucophanes between 315 ± 11 and 329 ± 33 Myr and on four phengites between 327 ± 10 and 339 ± 10 Myr. The concordance of these results led them to conclude that both minerals were formed at the same time about 335 ± 20 Myr ago during an episode of high pressure metamorphism.

Their data, however, as well as our results, show that phengites give systematically older ages than glaucophane. An independent measurement by H. Maluski[9] by the $^{40}\text{Ar}/^{39}\text{Ar}$ method has also confirmed the lower age of glaucophane (320 ± 8 Myr) compared with phengites. This difference is significant and consequently the ages of 320 ± 8 and 335 ± 20 Myr cannot represent the time of high pressure metamorphism.

This relation between glaucophane and cogenetic phengite has also been found by Coleman and Lanphere[10], Suppe and Armstrong[11] in the Californian blueschists and by Hunziker[12] in Alpine high pressure metamorphic terrains. These authors suggest that glaucophane has lost argon during later metamorphic or tectonic events, and that glaucophane ages may postdate high pressure metamorphism.

Such an interpretation seems to apply to the data from Groix; glaucophanes are often aligned parallel to early isoclinal fold axes, probably synchronous with high pressure metamorphism, but subsequently, they often became reoriented about axes of later folding and underwent recrystallisation. This suggests that 320 Myr glaucophane ages, in fact, record the effect of this later folding phase, and that the high pressure metamorphism occurred at, or before 375 Myr ago.

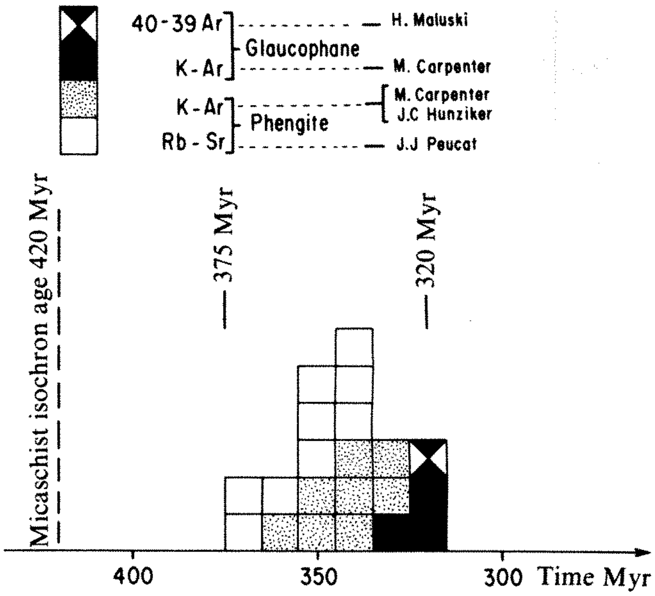

Fig. 1 Rb/Sr and K/Ar ages for phengites and glaucophanes. $^{40}\text{Ar}/^{39}\text{Ar}$ step release age for glaucophane.

Whole rock Rb–Sr isotopic data on four micaschists have been previously published by Vidal[13]. New measurements on two micaschists without glaucophane and on two with glaucophane as well as these four data of Vidal allow us to construct a reference isochron with an age of 420 Myr (Fig. 2).

Among the basic igneous rocks, one data point (Fig. 2) (an alkali-rich rock characterised by the presence of abundant phengitic mica: 2444) also lies near, or on, the micaschist isochron. However, most metabasaltic rocks have very low Rb/Sr ratio (<0.25) and as their $^{87}\text{Sr}/^{86}\text{Sr}$ ratios lie between 0.7052 and 0.7101, they do not allow construction of an isochron. Assuming an age of 420 Myr for the time of metamorphism, the calculated initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios range from 0.705 to 0.709. Reasons that might explain this scattering are: possibly seawater alteration effects, contamination from sediments, heterogeneous igneous source material with large differences in initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios. Preliminary chemical data on major and trace elements suggest that the original source rocks are produced from alkali-basalts and tholeiites, but higher initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios values suggest possible contamination.

We do not think that this age of 420 Myr reflects the sedimentation age because this would certainly have been disturbed by two metamorphic episodes[14,15]. Near Groix, very similar protoliths (Pouldu series) are cut by the Moëlan granite[16] dated at 474 ± 2 Myr (λ $^{87}\text{Rb} = 1.42\times10^{-11}\ \text{yr}^{-1}$) by Vidal[13]. Sedimentation here, therefore, pre-dates this Ordovician age.

Assuming that the 320 Myr age on glaucophane reflects the effects of a later metamorphic and deformation event, it is possible to associate this event with retrograde metamorphism. Consequently, the age of 420 Myr could reflect the high pressure–low temperature metamorphism, or a previous episode of metamorphism, or dewatering of sediments.

A pre-high pressure–low temperature metamorphism is possible, but is not recognised from petrographic study. When we take into account that the age 420 Myr is deduced from a reference isochron and that the 360–375 Myr mica ages are cooling ages, then the difference between these two ages does not necessarily imply two separate periods of metamorphism. Hence we interpret the age of 420 Myr as representing the time of high pressure–low temperature metamorphism, or possibly, the time of dewatering of sediments.

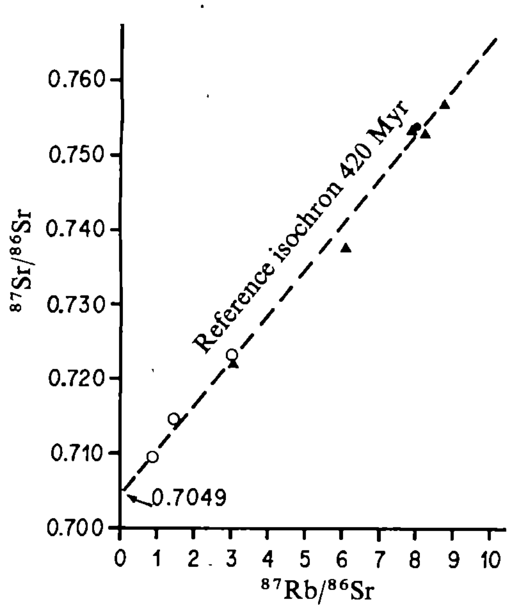

Fig. 2 Whole-rock reference isochron for micaschists: (○) micaschists with glaucophane, (▲) micaschists without glaucophane. Age calculated from seven points is 421±7 Myr with an ^{87}Sr/^{86}Sr initial ratio of 0.7049±3.10^{-4} (λ ^{87}Rb = 1.42×10^{-11} yr^{-1}). (●) Micaceous metabasic rock (2444).

In conclusion, the following metamorphic episodes can be delineated: (1) Pre-Hercynian period is characterised by the advent of high pressure–low temperature metamorphism about 370–420 Myr ago. The limits of this metamorphic period in South Brittany can be constrained by the study of Morbihan migmatites. The migmatisation affects an orthogneiss dated at 462±7 Myr by Peucat and culminates with emplacement of anatectic granites at 376±12 Myr (ref. 17). High pressure metamorphism and migmatisation events correspond with an important pre-Hercynian orogeny, possibly, with paired metamorphic belts of *circum* Pacific type, in which the basement was formed. (2) The Hercynian period produced partial retrogression of high pressure mineral asemblages into greenschist facies causing loss of argon in glaucophane. The continental basement was also affected by this retrograde metamorphism, especially near folds and shear zones. It is characterised by major plutonism *ca* 340–290 Myr ago. The thermal history of this area ends with cooling beneath 300 °C 290 Myr ago (Rb/Sr mica ages throughout south Brittany give 280–290 Myr) (ref. 18).

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^{40}Ar/^{39}Ar ages of a diabase sill and a basalt in the Central-Pacific Basin

The deep sea drilling project (DSDP) Leg 17 has covered the area of the Central-Pacific Basin south of the Mid-Pacific Mountains and east of the Marshall Islands. Since this region belongs to the magnetic quiet zone, no definite ages of oceanic crust could be assigned. Results of drilling at Site 166 (3°45.7′N, 175°4.8′W) and at site 167 on Magellan Rise (7°4.1′N, 176°49.5′W) have given estimates of crustal ages of about 120 and 135 Myr respectively from the basement fossils, which suggests that ages increase northward on this set[1]. Furthermore, the parallelism of linear bathymetric features around site 167 to the magnetic and bathymetric trends around site 166 has been interpreted by Winterer[1] to mean that they belong to the same spreading ridge system with an average spreading rate of 2–4 cm yr^{-1} for the crust between the two sites. Since sites 169 and 170 are located to the north-west of the sites 166 and 167 in the magnetic quiet zone, they were expected to give older ages, probably Jurassic, from the extrapolation of the trend stated above and by referring to the time scale of magnetic reversals[2]. It has been revealed, however, that at site 169 (10°40.2′N, 173°3.0′E) the oldest sediment recovered yields Albian age (100–104 Myr), which overlies basalt. At this site, about 8 m of diabase sill was drilled, which intruded Turonian (87–95 Myr) or Cenomanian (95–100 Myr) sediments[3]. At site 170 (11°48.0′N, 177°37.0′E), the oldest sediment overlied the basalt is also of Albian age[4]. These ages are much younger than those expected from ages obtained at sites 166 and 167, which may reflect the different history of the ocean floor from those at sites 166 and 167. To clarify this point, two igneous rocks were dated by ^{40}Ar/^{39}Ar method. Details of experimental procedures are described elsewhere[5].

The sample 169-6-2 is a diabase sill drilled at site 169 and relatively fresh among drilled samples, while the sample 170-16-2 is a basalt drilled at site 170, which was the lowermost one at this site. Results of ^{40}Ar/^{39}Ar dating are shown in Fig. 1*a* and *b*.

As shown in Fig. 1*a*, for the sample 169-6-2 the plot of the highest temperature fraction is clearly deviated to the higher ^{40}Ar/^{36}Ar ratio from the isochron, which indicates the existence of excess ^{40}Ar in this fraction. Excluding this point, however, other points form a good isochron of 90 Myr with the MSUM value of 1.428. This age is nearly

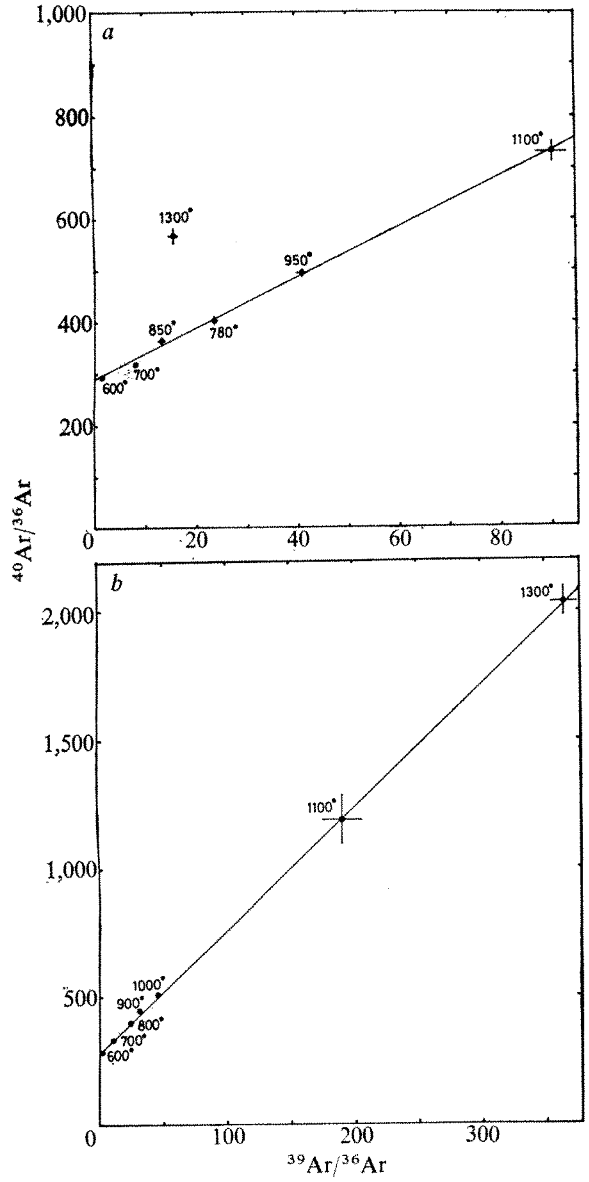

Fig. 1 ^{40}Ar/^{39}Ar isochron diagrams for Leg 17 samples. Samples were heated for one hour at each temperature which is indicated by figures at each plot. Horizontal and vertical bars at each plot indicate error range (one standard deviation) for each analysis. Bern 4M muscovite (18.5 Myr) was used as an age standard. (All necessary corrections including those for interference products have been made.) (*a*) Sample Leg 17–169–6–2; age: 90.0±3.5 Myr; intercept: 285.4±3.5, (*b*) Sample Leg 17–170–16–2; age: 97.2±2.5 Myr; intercept: 280.3±2.1.

the same or a little younger than that of intruded sediments. Petrologically the diabase has been regarded to have intruded as a sill rather than extruded as a flow[3]. Our results agree with this observation.

The ^{40}Ar–^{39}Ar isochron age of the basalt 170-16-2 is 97 Myr (Fig. 1*b*) and a little younger than that of the overlying sediments which contain late Albian radiolaria[4]. These basalts are reported to be altered[4], but stepwise-heating result has shown that it causes apparently no serious effect on the obtained age. The MSUM value for this sample is 1.605. Results for the sample 170-16-2 supports the middle Cretaceous age of basalt as estimated from the overlying sediments, which is much younger than Jurassic age which was expected. There are two possible explanations for this difference.

First, the basalt drilled at site 170 may be only a sill and not represent the true basement whose age is older than that obtained in this study. Bass *et al.* has interpreted the basalt as a sill of ocean island tholeiite from textural evidence and lack of glass or pillow structure[6]. In order to explain the existence of the magnetic quiet zone in this region, the crustal age should be in Jurassic and older than about 155 Myr, considering a long period of normal geomagnetic polarity at that time[2].

Secondly, the sample 170-16-2 may represent the basement rock, and the oceanic crust in this region might be really young—of middle Cretaceous age. Although the ^{40}Ar/^{39}Ar isochron age obtained for present sample is a little younger than that estimated for the overlying sediments, the difference may not be significant, considering its error range. Since a long period of normal geomagnetic polarity in Jurassic began about 108 Myr ago[2], the crustal age should be nearly the same or younger than about 108 Myr in order to explain the existence of the magnetic quiet zone. In addition to the age data, estimated from the lowermost sediments at sites 169 and 170, our present result is compatible with this interpretation. In this case, the crust in this region might be characterised by recurrent flows. In fact, many sills and/or dykes have been observed in the Mid-Atlantic Ridge area[7] and recurrent flows in the ocean floor may not be uncommon.

We have no preference between the two possibilities. Further deeper drilling is desirable in order to clarify the origin and formation ages of the Central-Pacific Basin.

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Mercury emissions from chlorine-production solid waste deposits

Before 1970 the amount of Hg discharged to the environment from anthropogenic sources was estimated to be comparable with quantities derived from continental weathering processes[1]. As a result, however, of several well-documented cases of acute Hg contamination throughout the world, most large industrial sources have been identified and the discharge of Hg reduced to negligible amounts relative to natural aquatic and atmospheric sources. The natural atmospheric dispersion mechanisms of Hg overshadow input into the global environment by human activity, the latter being of prime importance in localised environments. The role of the chlor-alkali industry in global and regional Hg cycling has been repeatedly discussed[2–5], with the general conclusion that their contribution may be of limited importance globally but of particular concern to local and regional water and air quality[6].

Although limited studies have been published on losses of Hg from production processes in active chlorine plants[3,4] none have considered the problem of atmospheric and aquatic emissions from waste products which have been ponded or otherwise stored near active or inactive plants. The study reported here concerns mercury emissions from wastes stored near an inactive chlorine plant.

The study area is at the site of a large chemical manufacturing facility in the eastern United States which ceased operation in 1972 because of an inability to meet newly imposed state and federal water quality standards. Sodium bicarbonate and related by-products were produced by the ammonia soda process since 1894. The plant began production of chlorine and caustic soda using the Hg-cell process in 1952. Between that time and 1972 waste products from both processes were disposed of in large sludge basins, a procedure relatively common in this industry[2,4,7]. The disposal site, with a surface area of ~ 44 ha, is situated adjacent to a river (daily mean discharge = 8.34 $m^3 s^{-1}$) in an area enclosed on one side by a natural ridge and on the river side by dikes built of $CaCO_3$ waste and boiler ash generated in the $NaHCO_3$ process.

We collected air, water, and waste samples within the study area during the period 1974–1976. Solid and aqueous samples for Hg determination were handled and analysed according to standard flameless AAS methods[8]. Air samples for particulate and vapour Hg were collected using standard air filter and activated charcoal absorption techniques[9,10] respectively, and analysed as above.

The volatility of metallic Hg (Hg°) and many of its compounds suggests its presence in large scale waste deposits such as these to be a potential threat to local air quality. Waste material collected from the surface of the waste ponds was used in laboratory experiments to determine the emission of Hg° from a known surface area at controlled temperatures. Approximately 4 kg of waste material (bulk material concentration = 155 μg Hg g^{-1}) was slurried into a 13-litre spherical glass chamber and allowed to dry to a moisture level similar to that observed for the surface of the actual waste ponds (approximately 75% water by weight). Mercury-free air was pumped through the chamber over the waste surface at 1 litre min^{-1} and the filtered output air sampled for Hg°. The emission of elemental Hg vapour from the waste material was clearly related to surface temperature (Fig. 1) as expected. The nature of the relationship is similar to that between temperature and the saturation concentration of Hg° in air[2,11] suggesting that the mercury occurs in the waste material to a large extent as the elemental species. Comparative data on Hg emission rates from natural soils are scarce, although some authors have reported observing release from soils experimentally enriched with inorganic Hg[12–14]. Natural degassing rates of Hg on regional or global scales have been estimated based on input/output calculations[15,16] as ranging from 0.02 to 0.03 μg $m^{-2} h^{-1}$, while measured rates for mineralised soils[17] are as high as 1.7 μg $m^{-2} h^{-1}$. We have recently measured the loss of Hg° from mercury-contaminated surface soils (total Hg = 99 μg g^{-1}) collected within 1 km of a large active Hg mine, and found emission rates at 33 °C to be in the range of 1.4–1.7 μg $m^{-2} h^{-1}$. These values are two orders of magnitude lower than those we have measured in comparable conditions for the waste material (120–170 μg $m^{-2} h^{-1}$). The difference is probably related to the form of Hg present in the two samples; the mine site soil contains primarily HgS and HgO.

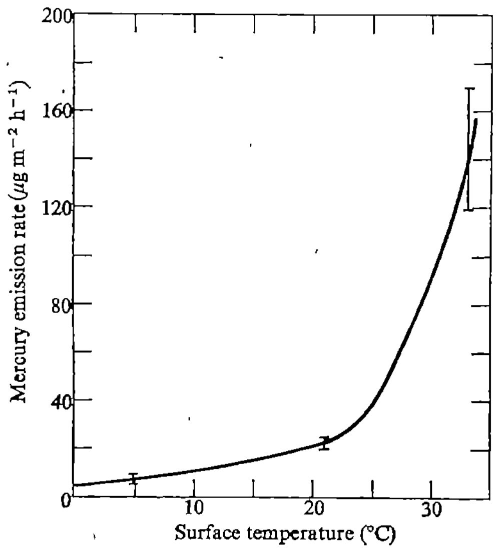

Fig. 1 Relationship between emission of vapour phase elemental mercury and surface temperature of waste material. The numerical relationship derived from a nonlinear least squares fit is $Y = a + b\exp(cX)$, where Y = emission rate in μg $m^{-2} h^{-1}$, X = surface temperature in °C, a = 6.40 (standard error = 1.59), b = 0.310 (standard error = 0.100), c = 0.190 (standard error = 0.00982).

Table 1 Daily mean concentrations of mercury vapour (Hg°) in air in vicinity of waste ponds under various meteorological conditions

Distance from centre of waste ponds (km)	Time downwind (%)*	Mean air temperature (°C)	Concentration of mercury vapour (Hg°) in air (ng m^{-3}) Measured	Predicted
0.4	78	6.3	90	65
0.5	99	6.3	64±6†	72
1.5	67	6.3	18	26
1.9	21	6.3	12	15
0.5	96	29.4	991±2†	778

*Expressed as a percentage of the total sampling period (24 h) during which the sample location was downwind of the waste pond.
†Replicate samples.

Considering the waste deposits as an area source, we have applied a simple Gaussian diffusion model[18] to estimate 24 h mean concentrations of Hg vapour in the ambient air downwind of the source. The emission rates measured in controlled conditions (Fig. 1) were used in the model; mean wind speed directly over the pond area was assumed to be 1 m s^{-1}; the background air Hg concentration was taken as 10 ng m^{-3} (ref. 15). Given the simplicity of this approach, the close agreement between measured and predicted 24 h mean air concentrations in similar conditions (Table 1) can be taken as an indication that the entire surface area of the waste pond was emitting Hg vapour at a rate comparable to that measured in controlled experiments.

The levels of Hg° in air measured near the pond during periods of low air temperature (Table 1) were slightly elevated above those reported for large US cities[3,18]. The fact that these concentrations decreased to rural background levels[3] within 1.5 km of the waste disposal area is attributable to the ability of a well mixed atmosphere to dilute gaseous pollutants rapidly. However, during periods of higher air temperature, the concentration of Hg° measured near the pond area approached the US Environmental Protection Agency suggested standard for total Hg in ambient air (1,000 ng m^{-3}) (ref. 19). This compares with levels of 30–1,600 ng m^{-3} for air over natural Hg deposits and 10–28,000 ng m^{-3} for natural degassing and geothermal emissions[3]. The concentration of particulate Hg during this sampling period was surprisingly low (0.25–0.36 ng m^{-3}) considering the area in question, and reflected the stability of the waste pond surface to dust resuspension. The indication that essentially 100% of the Hg in air is in vapour form agrees with recently reported results[19].

The location of the waste ponds behind steep dikes along a riverbank and perched on the flank of a ridge is highly conducive to the leaching of soluble components of the waste by direct rainfall and by runoff from the adjacent ridge. Clay tiles installed beneath the dikes serve to drain the waste from the bottom while overflow structures drain the ponds from the surface and intermediate depths. Discharge of waste leachate

Table 2 Summary of determinations of mercury fluxes from chlor-alkali plant waste disposal pond area using mass balance approach

Upstream*			Downstream†			
Total Hg ($\mu g\ l^{-1}$)	Stream discharge‡ ($m^3\ s^{-1}$)	Total Hg flux ($g\ d^{-1}$)	Total Hg ($\mu g\ l^{-1}$)	Stream discharge‡ ($m^3\ s^{-1}$)	Total Hg flux ($g\ d^{-1}$)	Net Hg flux ($g\ d^{-1}$)
0.077	1.76	11.7	0.220	1.98	37.6	25.9
0.006	1.70	0.88	0.167	1.92	27.7	26.8
0.007	1.61	0.98	0.185	1.81	28.9	27.9
0.008	1.57	1.09	0.193	1.77	29.5	28.4§
0.052	2.83	12.7	0.186	3.19	51.3	38.6
0.012	6.00	6.22	0.116	6.80	68 2	62.0
0.003	11.5	2.98	0.094	12.2	99.1	96.1
0.002	13.1	2.26	0.087	14.8	111	109
0.005	14.5	6.26	0.084	16.3	118	112
0.008	19.1	13.2	0.123	21.5	228	215
0.015	26.7	34.6	0.103	30.1	268	233
0.012	18 2	18.9	0.209	20.6	372	353

*2.7 km above chlor-alkali plant.
†6.4 km below chlor-alkali plant.
‡Based on discharge recorded at nearby US Geological Survey Stream Gaging Station and adjusted for difference in drainage area.
§Measured flux on this day was 14.3 $g\ d^{-1}$.

through these structures and many intermittent seepages flow directly into the adjacent river. Our own direct measurements of leaching rates of Hg from the waste area were limited to periods of very low river discharge, since most of the tile drains were submerged and thus inaccessible for sampling and flow measurement during higher flows. Consequently, we also applied a mass balance approach to estimate net fluxes of mercury to the adjacent river (Table 2) Using mercury concentrations and stream discharge measurements taken between 1974 and 1976 at points immediately upstream and downstream of the waste pond area[10], we calculated net fluxes of mercury as the difference between upstream and downstream fluxes. To the extent that mercury accumulates in river bed sediments during periods of low river discharge, or is flushed from river bed sediments during periods of high river discharge, mass balance calculations based on instantaneous measurements will either underestimate (at low discharge) or overestimate (at high discharge) the actual leaching rate of mercury from the waste ponds. Direct measurements of all accessible flows of waste leachate into the adjacent river during a period of low river discharge offered some reasonable verification of a mass balance calculation made for the same period. On one day the total measured flux of mercury was 14.3 $g\ d^{-1}$ while the calculated net flux was 28.4 $g\ d^{-1}$. The lower measured flux probably results from our inability to locate and measure all leachate seepages rather than from an overestimate by the mass balance calculation.

Our estimates of aquatic fluxes of mercury from the waste pond area indicate considerable variability in the apparent leaching rate of mercury from the waste, but demonstrate a good correlation ($r = 0.86$; $P < 0.01$) with discharge of the adjacent river which responds primarily to local rainfall in the upstream 575 km^2 catchment. However, it is not clear whether this correlation results from a direct dependence of mercury leaching rate on rainfall input or from the dependence of bed sediment erosion on river discharge. Both processes probably contribute to higher net mercury fluxes from the waste pond area during periods of higher river discharge.

Using simple regression analyses we have obtained functions which estimate both surface emission of Hg°, based on air temperature, and aquatic leaching of Hg, based on streamflow. Applying these relationships to daily streamflow and hourly temperature data for the waste pond area for a typical year (October 1973–September 1974), we have calculated the estimated annual loss of mercury from the waste disposal site by both the atmospheric and aquatic pathways. Losses were nearly equally divided between these pathways $36\pm4\ kg\ yr^{-1}$ (predicted sum±the estimated standard error of the sum of predicted fluxes) atmospheric emission of Hg and $39\pm2\ kg\ yr^{-1}$ aquatic emission of Hg. The occurrence of high streamflow during cold winter months resulted in predominance of the aquatic pathways during winter and predominance of the atmospheric route during summer months. This results in a relatively uniform emission of Hg from the waste deposits over an annual cycle.

The estimated total annual flux of Hg from this inactive plant, $75\pm5\ kg\ yr^{-1}$, is approximately 6% of the US Environmental Protection Agency standard for emission of Hg to air and water from active chlorine plants situated in riverine environments. Recently reported losses of Hg to the atmosphere and surface waters by Swedish chlor-alkali plants were, however, in the range of 150–1,400 $kg\ yr^{-1}$ (refs 2, 6). Values for US plants are estimated to range up to 1,100 $kg\ yr^{-1}$ (ref. 3). Estimates of the capability of improved technology to reduce Hg emissions from active plants indicate a possible 40% decrease in emissions by these routes[3], resulting in Hg losses from the more efficient operations (that is, those reported above for Swedish plants) which would be similar to those reported here for waste disposal ponds alone.

At present the most significant mercury waste problem in this industry involves disposal of large quantities of solid waste materials. Thus, given the common disposal practice of ponding solid wastes, along with the residence time of Hg in existing large scale waste deposits (conservatively estimated as 100 years for the waste area studied here)[10], it becomes apparent that the role of such residual waste deposits in local Hg cycles will continue to grow in relative importance.

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Dissolved aluminium concentration in sea water

DISSOLUTION of clay minerals in seawater results in aluminium concentrations in solution (1.3–7.5 μg Al per l) which are similar to those found in coastal waters but considerably higher than the probable value of the aluminium concentration in open ocean surface waters (0.5 μg Al per l). This note compares the results of precipitation and dissolution and investigates the mechanism of the dissolution of aluminium from three different clay minerals. Our results show that bottom and suspended clay sediments probably act as a source of dissolved aluminium to sea water, whereas removal below clay solubility predictions is probably the result of biological activity, as is the case for silicon, the other main constituent of most rocks and minerals.

Recent results from the North Sea[1] and the North Atlantic[2] show that away from the influences of aluminium-rich freshwater sources and higher coastal concentrations of suspended matter the concentration of aluminium in seawater will be approximately 0.5 μg l^{-1}. This level is more than an order of magnitude lower than that predicted by alumina–silica coprecipitation experiments[3], which showed that at levels of silica which occur naturally in seawater, the solubility of aluminium is much reduced below that which is stable in silica free seawater. These experiments[3] may have produced erroneously high levels due to the inclusion of particulate aluminium in the analysis results and the failure of the experiments to reach complete equilibrium.

We carried out the following experiments (a fluorometric analysis method was used which is insensitive to aluminium in particulate matter[4].) The fractions of the clays of less than 2 μm were separated by centrifugation, and suspensions containing 10 g of clay in 1 l of sea water were prepared in polythene bottles. The *p*H of the suspensions were adjusted to *p*H 8.1 with a few drops of sodium carbonate solution. They were then maintained at 20 °C in the dark and continually shaken for four months. Aliquots were withdrawn periodically, centrifuged and filtered through 0.1-μm membrane filters before the aluminium and silicate analyses were carried out. The clays and seawater reached a steady state within three months. No trends are discernible in the measurements made at three and four months. After four months the *p*H of the solutions were readjusted to *p*H 8.1 using dilute sodium hydroxide, and maintained at this *p*H by daily control for one week before the aluminium and silicate concentrations were measured. The conditions were again changed by lowering the temperature to 3 °C and the new conditions were maintained for one week before the analyses were carried out. The results of this experiment are summarised in Table 1.

The pattern of silica dissolution followed that observed in previous studies[5]: a rapid initial rise in the concentration was followed by a more gradual increase over several weeks. The change in aluminium followed a completely different path after the initial sharp increase. For each of the three clays the highest aluminium concentrations were measured in the first aliquot to be withdrawn. The concentrations then fell gradually reaching apparent equilibrium in two months. To see to what extent the fall in aluminium concentrations observed was due to changes in *p*H, a second experiment was carried out using kaolinite, the *p*H being maintained at *p*H 8.1. The results of this experiment are shown in Fig. 1. The decrease in aluminium concentrations observed in the first experiment were not simply due to *p*H changes. Also, there was no evidence for a simple control of the aluminium concentration by silicate as the aluminium concentration continued to decrease after the maximum silicate concentration was reached. The plot of aluminium concentration against $t^{1/2}$ is approximately linear which suggests the rate controlling step is a diffusion process, which is amenable to further study.

When the results of these experiments (1.3–7.5 μg Al per l) are compared with those for precipitation (8–15 μg Al per l) (ref. 3) we see that dissolution, as would be expected for approaching equilibrium from the opposite direction, gives slightly lower values than precipitation. Both sets of results predict much higher concentrations of aluminium in seawater containing an average amount of silica than have actually been observed in open seawaters. In coastal waters aluminium concentrations of the predicted magnitude have been recorded (maximum levels observed in refs 1 and 2 are 5.5 and 4.6 μg Al per l respectively). The inference from comparing the results for open seawater and coastal seawater is that away from the higher suspended matter concentrations in coastal waters, the clay concentration is not sufficiently great to maintain the aluminium concentration in competition with biological activity which is removing aluminium from the water. A number of pieces of evidence support the idea of aluminium being removed from solution in some part of the life cycle of siliceous diatoms. Diatom frustules are known to contain aluminium[6], the source of which is uncertain. Possible montmorillonite structures have been observed in the frustules from living diatoms thought to be uncontaminated by detrital clays[7]. This suggests that aluminium

Table 1 Summary of results of first clay* dissolution experiment

Result†	*p*H	*T* (°C)	Kaolinite Al (μg l^{-1})	Kaolinite SiO$_2$ (mg l^{-1})	Fireclay Al (μg l^{-1})	Fireclay SiO$_2$ (mg l^{-1})	Montmorillonite Al (μg l^{-1})	Montmorillonite SiO$_2$ (mg l^{-1})
(1)	8.1	20	13.8	3.8	12.5	2.8	5.4	5.4
(2)	—	20	2.7	5.6	6.0	3.2	1.3	12.2
(3)	—	20	2.7	5.6	5.9	3.2	1.3	12.4
(4)	8.1	20	7.3	4.3	7.5	2.6	4.8	6.8
(5)	8.1	3	3.1	3.5	5.3	2.1	3.0	5.3

*Clays used: Kaolinite (A.P.I. H7), Fireclay (Glashafenton 1), Montmorillonite (Tixoton). Seawater collected 26 Jan. 1976 from N. Atlantic 59°4′N, 02°50′W. Initial concentrations: Aluminium 0.3 μg Al per l, Silica 0.1 mg SiO$_2$ per l.

†Results: (1) after 36 h; (2) after 3 months; (3) end of 4 months, final *p*Hs—Kaolinite 7.35, Fireclay 7.69, Montmorillonite 7.35; (4) after 4 months plus 1 week at *p*H 8.1; (5) after 4 months plus 1 week at *p*H 8.1 and 1 week at *p*H 8.1 and 3 °C.

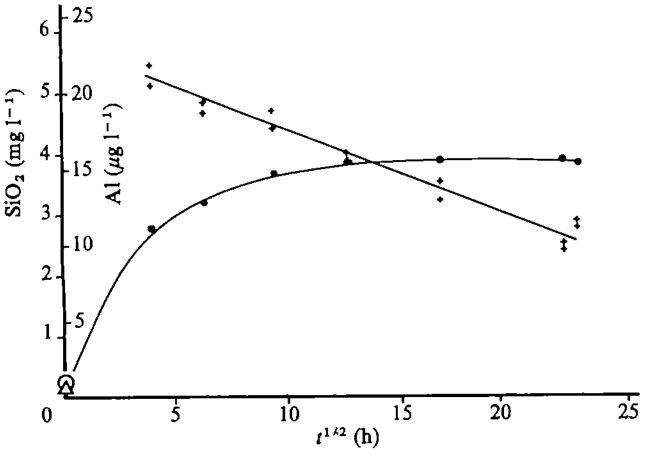

Fig. 1 Rate of dissolution of silica (●) and aluminium (+) from duplicate suspensions of kaolinite, 10,000 mg l^{-1} in sea water at pH 8.1 and 19±1 °C. For silica the difference between the samples taken is smaller than the data point. Initial concentrations of: ○, silica 0.1 mg SiO_2 l^{-1}; △, aluminium 0.3 μg Al l^{-1}.

removal may be an active biological process as the removal from biologically productive waters seems to be for other trace metals[8,9]. However, evidence also exists for the formation by adsorption on to the frustules of dead diatoms of alumino-silicate phases[10]. Further work is required to establish how strongly aluminium is adsorbed by siliceous diatom remains and what the balance is between that and active biological removal.

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Optimal foraging in great tits (*Parus major*)

OPTIMAL foraging theory[1-5] is based on the supposition that animals have been designed, by natural selection, to behave in a way that will maximise their inclusive fitness and that in terms of foraging this goal may be approximated by maximising the net rate of energy intake while feeding[1-4]. Therefore, given the constraints of any particular situation it is possible to predict how an animal ought to behave while foraging. I have tested an optimal foraging model which predicts how a predator should exploit patchily distributed prey. The results are consistent with the predictions of the model.

The prey of most predators are not scattered randomly throughout the environment[6] but occur in aggregations, which I call patches. To exploit these patches efficiently a predator needs to 'decide' which patches to visit and when to move from one to another. If the quality of a patch cannot be predicted in advance the problem simply becomes: how long is it best to remain in each individual patch? Several authors have produced similar models describing the optimal solution to this problem[7-9]. Owing to depletion of prey, the capture rate within a patch is likely to be a decreasing function of the time spent there[10]. This means that if a predator stays for too long its capture rate will fall below that which could be achieved by moving to the next patch. Conversely, if it does not stay for long enough it will spend too large a proportion of its time travelling. The optimal solution is that the predator should move when its capture rate within a patch drops to a threshold equal to the average capture rate for the environment as a whole. This critical threshold is called the marginal capture rate[7] (Fig. 1).

The optimal solution (Tp(opt)) is affected by changes in either the curve $f(Tp)$ or the time taken to travel between patches (Tt). In my experiments I manipulated the latter and kept the former constant so that it is possible to define the relationship between Tp(opt) and Tt as follows.

Assuming the curve $f(Tp)$ is an exponential of the form

$$f = Q[1-\exp(-mTp)] \qquad (1)$$

(where f is average net energy intake; Tp is time in patch; m and Q are constants), the rate of food intake after a length of stay Tp is given by

$$\mathrm{d}f/\mathrm{d}Tp = Qm\exp(-mTp) \qquad (2)$$

The optimal predator should leave a patch when its capture rate falls to the average capture rate for the environment[7,8]. That is when

$$\mathrm{d}f/\mathrm{d}Tp = f/(Tt+Tp(\mathrm{opt}))$$

where Tt is mean travel time.

Therefore

$$Qm\exp(-mTp(\mathrm{opt})) = f/(Tt+Tp(\mathrm{opt})) \qquad (3)$$

Substituting equation (1)

$$Qm\exp(-mTp(\mathrm{opt})) = Q[1-\exp(-mTp(\mathrm{opt}))]/(Tt+Tp(\mathrm{opt})) \qquad (4)$$

Rearranging this gives

$$Tt = 1/m[\exp(mTp(\mathrm{opt}))]-1)-Tp(\mathrm{opt}) \qquad (5)$$

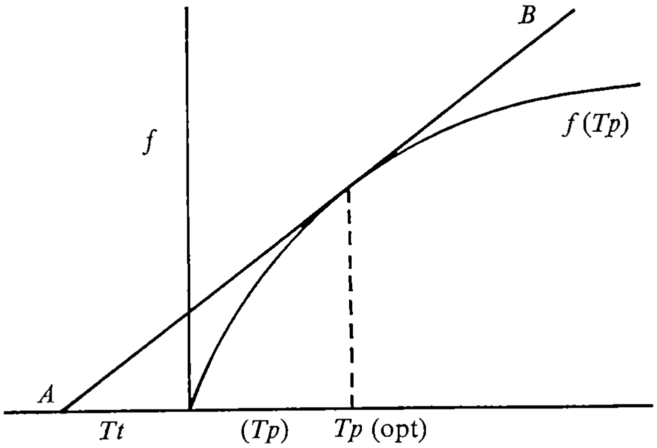


Fig. 1 Graphical representation of a model of optimal patch use[7,8]. The curve $f(Tp)$ represents the average net energy gain (f) as a function of the time spent in each patch (Tp). The abscissa is extended in the negative quadrant to a distance Tt which is the mean time taken to travel between patches. The maximum average net rate of food intake that is obtainable can be determined by constructing the tangent to $f(Tp)$ from point A. The predator ought to leave each patch after time Tp(opt) in order to maximise its average capture rate.

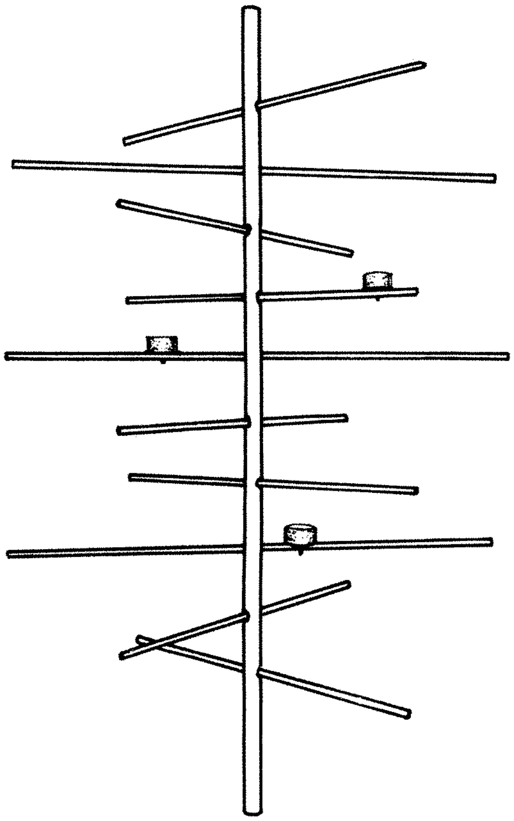


Fig. 2 An experimental 'tree'.

This relationship makes it possible to test the predictions of the model quantitatively.

I performed the experiments on six wild caught great tits (*Parus major*). The apparatus consisted of an aviary (4.6 m × 3.7 m), containing five artificial trees (Fig. 2). Each tree supported six patches. Patches were made from 4-cm lengths of plastic drainpipe (6.5 cm diameter), which were sealed at one end. The patches were filled with sawdust in which I hid the prey, six quarter sections of mealworm.

I subjected each bird to two types of 'environment' which were identical in all respects except for the time and energy cost of travelling between patches. Ideally these costs should have been increased by placing the patches farther apart but it was impossible to separate them by large enough distances within the confines of the aviary. Instead, I covered each patch with a cardboard lid which the bird had to remove before it began foraging. There were two types of lid. One rested on top of the drainpipe and could easily be flipped off, the other fitted just inside the rim and had to be prised out. Each experimental environment contained patches with lids that were either all 'hard' or all 'easy' to remove, these differences corresponding to long and short travel times respectively.

I performed six trials on each bird in each environment. Every trial was preceded by 2 h of food deprivation and was terminated when the bird had foraged for 10 min. Satiation and overall depletion of the environment had no observable effect within this period. Throughout each trial I watched the bird from behind a one-way mirror and recorded its behaviour on a Dawkins' behaviour organ[11].

Table 1 shows that the changes in travel time have a significant effect on the average capture rate and the average time spent in each patch. To calculate $Tp(\text{opt})$ from equation (5) it is necessary to know the formula for the curve $f(Tp)$ (Fig. 1). I determined this curve by calculating the mean number of prey caught as a function of the time spent searching in a patch. For each bird the best fit was provided by an exponential of the form described previously (equation (1)). Figure 3 shows the actual relationship between the mean travel time and the mean time spent per patch, for each bird in both environments. The dotted curve is the optimal solution calculated using equation (5) and the mean $f(Tp)$ curve for all six birds. Most of the data points lie above this curve which indicates that the birds tended to stay in each patch for too long. This can be explained by the fact that the curve $f(Tp)$ only takes into account the average gross rate of energy intake. If adjustments are made for the energetic costs of travelling and searching the relationship in equation (5) can be rewritten as

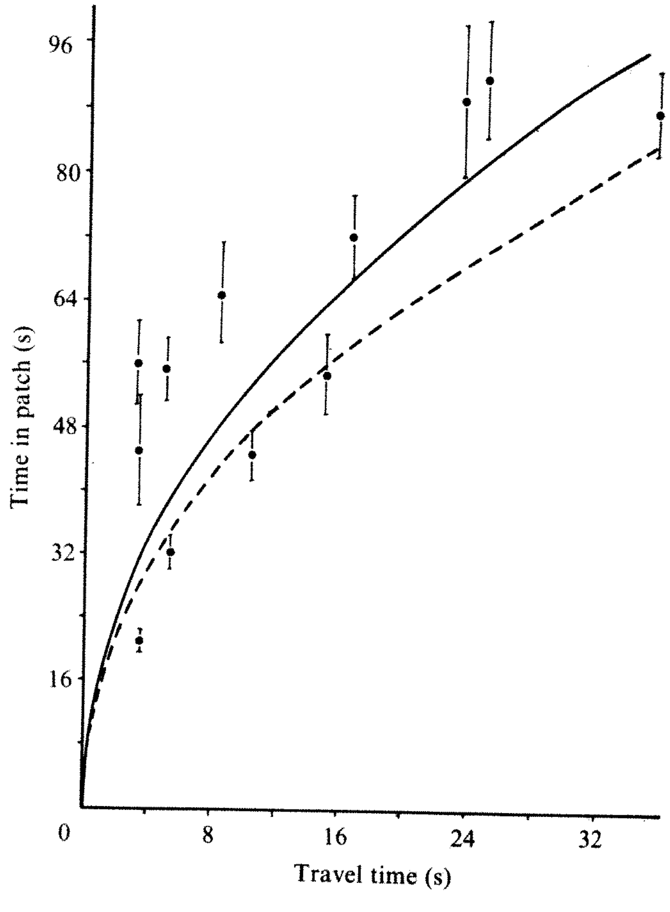
$$Tt=[KQ(1-\exp(-mTp)(1+mTp))]-P/(KQm\exp(-mTp)-B)$$

Table 1 Summary of principal variables in each environment

Variable	'Hard' environment	'Easy' environment	Paired t value	Significance level, 5 d.f.
Mean intercatch interval (s)	15.37 (1.37)	14.41 (1.87)	0.98	NS 2-tailed
Time in patch (s)	73.67 (8.06)	45.80 (6.77)	2.95	$P < 0.02$ 1-tailed
Handling time (s)	6.79 (0.60)	9.62 (1.65)	1.51	NS 2-tailed
Travel time (s)	21.03 (3.65)	4.76 (0.80)	4.73	$P < 0.002$ 1-tailed
Average capture rate (prey per s)	0.0264 (0.00277)	0.0366 (0.00541)	3.52	$P < 0.02$ 1-tailed

Averages of data for all six birds. The figures in brackets are standard errors. The average capture rate was determined by dividing the total number of finds in each trial by the total foraging time. The mean intercatch interval within a patch is the average time spent searching for each prey item. Handling time is the time taken to manipulate and eat a prey item. Time in patch and travel time are explained in the text.

Fig. 3 The relationship between the mean travel time and the mean time spent in each patch, for each bird in both experimental treatments. The vertical bars represent the standard error. The dotted curve shows the optimal solution derived from equation (5) and the unbroken curve shows the optimal solution after making adjustments for energy expenditure. $P = 0.697$ cal s^{-1}; $B = 0.155$ cal s^{-1}; $k = 56$ cal; $Q = 6.3587$; $m = 0.00813576$.



where P is energy cost of travel (cal s^{-1}), B is energy cost of searching (cal s^{-1}), K is calorific content of prey (cal). The basal metabolic rate (BMR) of a great tit was calculated using the equation of Aschoff and Pohl[12]. Estimates of the energy requirement for flight and feeding were taken as 9 × BMR and 2 × BMR respectively[13,14]. The solid curve in Fig. 3 shows the optimal solution based on net energy intake. It is not significantly different from the observed data. (Paired comparisons $t = 1.65$ ($t_{0.05} = 2.20$).)

These results show that great tits can exploit patchily distributed prey in a manner predicted by optimal foraging theory. Previous attempts to test this type of model[15,16] have been based on qualitative predictions, whereas I tested a quantitative relationship. The results imply that the birds can estimate both their instantaneous rate of intake within a patch and the average rate of energy intake over their environment as a whole. Unfortunately this concept of 'environment' is ill defined and it is difficult to know over what sort of time or area the birds might be averaging. One possible solution is that they have a memory window which produces a sliding average over the last few patches or minutes. In a constant environment a large window would result in an accurate measure of the average capture rate. In a fluctuating environment, however, a very large window could result in the predator adapting too slowly to changes in prey density. It seems likely that there is an optimal size for this memory window, and experiments to determine its size in great tits are underway.

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Nature and precursors of juvenile hormone-induced excessive cuticular melanisation in German cockroach

GROWTH in insects proceeds through a number of moults, when the old cuticle is shed and a new one synthesised. Within few hours of ecdysis, the cuticle hardens and develops the characteristic colour pattern. When we treated German cockroach *Blattella germanica* (L.) nymphs with insect juvenile hormone (JH) 12 h before ecdysis, the resulting nymphs or adults developed excessive black coloration all over their bodies, obliterating the normal colour patterns[1]. The nature of this visible change was unknown[2,3]. Here we show for the first time that the excessive coloration, following JH treatment, is caused by melanin, and that dopamine or catechol seems to be involved in the hormone-influenced pathway.

The striped pattern of the dorsal plate (pronotum, Fig. 1) provided ready identification of melanised cockroaches. When a melanised nymph moulted into an older nymph or adult, the abnormal coloration was lost together with the old cuticle. Since only the outer layers of the integument are shed at the moult, this meant that the dark pigment was contained in these outer layers and not in the underlying epidermis. Cross sections of the cuticles (Fig. 2) confirmed that the pigment was located in the exocuticle, and that it was present even in areas that are normally devoid of pigment. We isolated the black pigments from the normal and melanised cuticles and studied their infrared spectra in comparison with that of synthetic melanin (Fig. 3). We found the cuticular black pigments to be quite comparable with the synthetic melanin and that the spectra from normal and melanised cuticles were identical. We therefore determined the amounts of melanin in the normal and melanised cuticles fluorimetrically[4], and found that the melanised cuticles contained 6.5 mg melanin (per g dry cuticle) compared with 4.8 g in the normal cuticles—a 35% increase.

We then proceeded to identify the precursors that might be on the pathway to excessive melanin biosynthesis. We

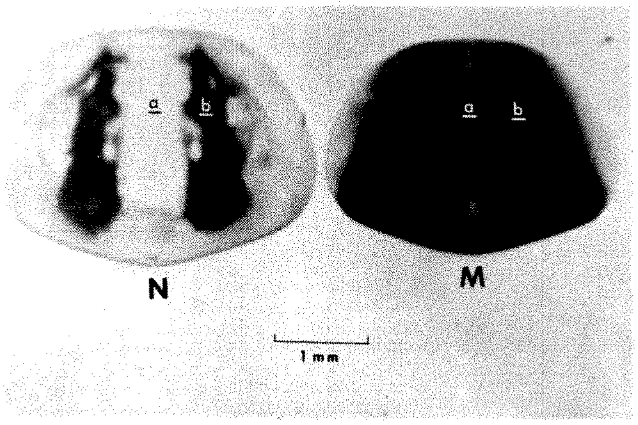
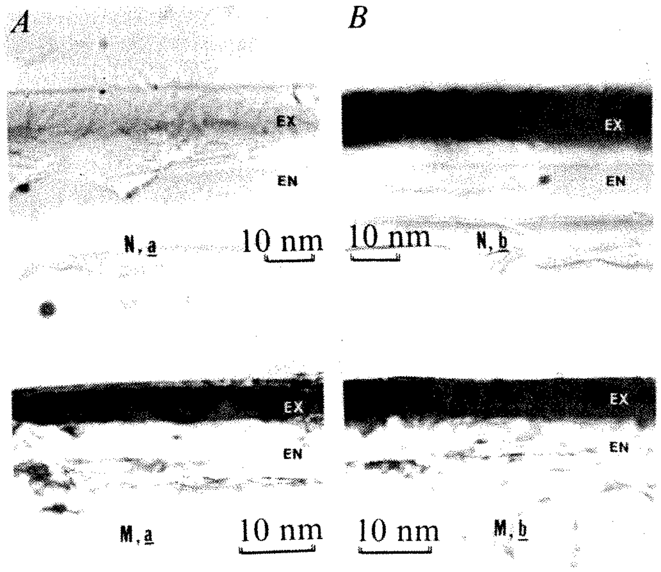


Fig. 1 Pronota of normal (N) and melanised (M) *Blattella germanica* adults showing the non-melanised (*a*) and melanised (striped, *b*) zones in normal adult and the corresponding zones in melanised adult. Synthetic *Cecropia* juvenile hormone, methyl-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate, (or any of its mimics[8]) was applied topically (1.5 μl), by injection (0.33 μl), or by treating the food (200 p.p.m.).

Fig. 2 Cross section of pronota of normal (N) and melanised (M) *Blattella germanica* adults; 10 μm, unstained. The clear zones (*A*) and striped zones (*B*) of the normal and melanised pronota are compared. Note the presence of melanin in zone *a* of melanised cuticle obliterating the normal melanic pattern. EN, Endocuticle; EX, exocuticle.



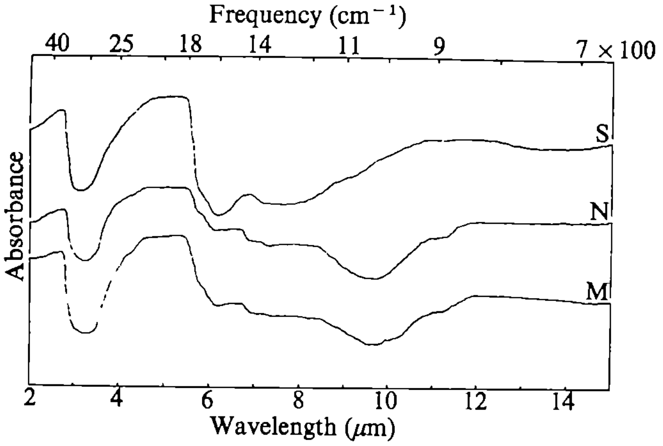


Fig. 3 Infrared spectra of synthetic melanin (S) and cuticular melanins of normal (N) and melanised (M) *Blattella germanica* adults using KBr disks containing 1% melanin. Dried cuticles were hydrolysed in acid and melanin was extracted in alkali[9-11].

incubated the fresh, soft, untanned cuticle (with the underlying epithelium intact) from normal cockroach in a tissue culture medium[8] modified by deleting tyrosine, tryptophan, phenylalanine, and foetal calf serum. Treated media were prepared by selectively incorporating the substrates and/or inhibitors into the culture medium—all at a final concentration of 3×10^{-3} M and *p*H 7.0. The media were oxygenated for 5 min through a fine capillary tube before introducing the cuticle. After incubating in the media for 5–10 h at 35 °C, the cuticles were washed in 95% ethanol and the coloration observed. The presence of tyrosine alone was adequate for the development of normal colour pattern. This could be inhibited, however, by either diethyldithiocarbamate (an inhibitor of tyrosinase[6], EC 1.14.18.1) or 1-(DL-seryl)-2-(2,3,4-trihydroxybenzyl) hydrazine HCl (an inhibitor of dopadecarboxylase[7], EC 4.1.1.28), showing that the cuticle normally contains all the required enzymes for melanin biosynthesis. Also, there was no need for JH in the medium. If either dopamine or catechol was provided in lieu of tyrosine, there was excessive melanisation similar to that observed in the JH-treated individuals. It is possible that the exogenous JH interferes with the timely autoinhibition of tyrosinase *in vivo* leading to unrestricted melanisation. Alternatively, a more readily utilisable precursor like dopamine is made available to the integument by induced synthesis, activation of stored molecules, or increased transportation. Absence of JH, which is generally the case at the time of moulting, seems to be a natural prerequisite for the normal colour development.

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Simultaneous recording from several neurones in an invertebrate central nervous system

THE ability to monitor activity in many neurones simultaneously might provide a powerful tool for solving certain problems in neurobiology. There are several changes in the optical properties of a neurone that occur during an action potential[1-3] that might be used in an apparatus that could monitor spike activity in many neurones simultaneously. Although the changes in intrinsic (native) optical properties have been useful in other instances where an optical measurement of membrane potential was desired, larger signals were needed for this application. Therefore, a search was begun for extrinsic probe molecules which could serve as sensitive molecular transducers in stained membranes, transforming changes in membrane potential into changes in light intensity[4,5]. In earlier experiments we demonstrated changes in fluorescence[6] and absorption[7] during action potentials in individual neurones of a segmental ganglion from the leech, *Hirudo medicinalis*. In those experiments the ganglion was stained with a merocyanine dye[8]. In the experiments described here, carried out on the supraoesophageal ganglion of the barnacle, *Balanus nubilus*, the absorption change of a new merocyanine-oxazolone dye[9,10] was used. In measurements on squid giant axons, the signal obtained with the new dye was five times larger. In addition, the photodynamic

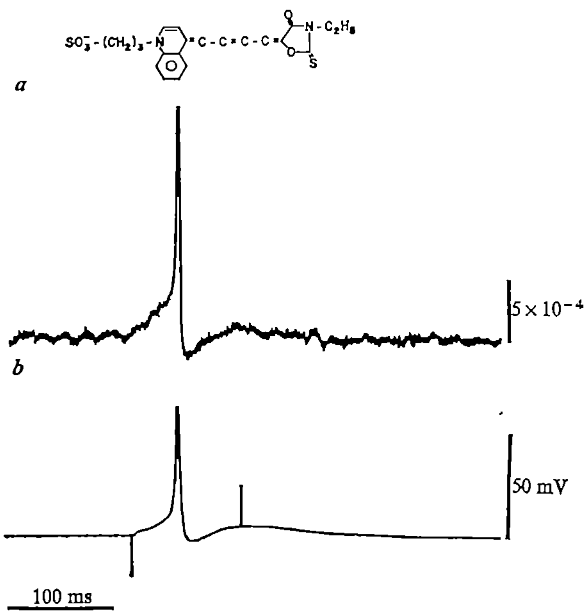

Fig. 1 The change in absorption (*a*) during an action potential (*b*). The structure of the merocyanine-oxazolone dye used in these experiments is shown at the top. The action potential size and time course were unaffected by turning on the light; no photodynamic damage was detected in the barnacle experiments even though the saline was equilibrated with air. The direction of the arrows to the right of the optical traces indicates an increase in absorption and the length of the arrow represents the stated value of the change in absorption divided by the resting absorption. An interference filter with peak transmission at 720 nm and width at half-height of 30 nm was positioned between the light source and condenser lens to restrict the measurement to wavelengths where the signal was largest in squid experiments. The output of each photodetector was amplified, limited to a bandpass of 1 Hz–0.3 kHz, and then displayed on a storage oscilloscope; permanent records were made with a Polaroid camera. The records were traced from enlargements of the original photographs. The ganglia were pre-incubated with a 1.5 mg ml^{-1} solution of collagenase in barnacle saline[11] for 5 min. The experiments were carried out at room temperature, 20–23 °C.

damage[8,11] associated with photochemical effects of intense illumination in the presence of dye and oxygen was 100 times smaller with the new dye.

To measure the absorption of light by individual neurones, an enlarged image of the ganglion was formed with a ×20, 0.4 n.a. objective using a Leitz Ortholux II microscope. Light guides (Schott, LST, clad glass rod) were used to carry the light from images of the individual neurones to individual photodiodes (PV 100A, E.G. & G. Inc.). The light guides and photodiodes were fixed to magnets on a stainless steel annulus above the image plane. The tips of the light guides had diameters of 1.5 mm and were positioned over the images of neurones by moving the magnets. The sizes of the large neurones on the dorsal surface of the ganglion were between 40 and 90 μm.

The barnacle ganglia were incubated for 20 min with a 1 mg ml^{-1} solution of the merocyanine-oxazolone dye shown in Fig. 1 (available as NK 2367 from Nippon Kankoh-Shikiso Kenkyusho, Okayama, Japan). The dye solution was then replaced with saline and an individual neurone impaled with a microelectrode. The tip of a light guide was positioned over its image, and a depolarising current was then passed through the microelectrode to stimulate the neurone. A simultaneous optical and electrode measurement is shown in Fig. 1. The change in intensity at the peak of the action potential divided by the resting light level was only about 10^{-3} and was often smaller. Even so, the signal-to-noise ratio in Fig. 1 is greater than 10 : 1. But, it was only after considerable effort[13] that we were able to reduce the noise from mechanical vibrations and other optical disturbances so that most of the noise in Figs 1 and 2 results from random arrival of photons at the photodetectors (shot noise[14]). Significant improvements in the signal-to-noise ratio can be achieved only by modifying the apparatus to increase the intensity reaching the photodetectors or by finding more sensitive dyes.

The signal-to-noise ratio illustrated in Fig. 1 was large enough so further experiments were carried out to determine whether synaptic potentials could also be detected optically. In neurones with excitatory or inhibitory synaptic potentials of 10 mV or greater, we could reliably detect the synaptic events in the optical trace. In the best circumstances, synaptic potentials of only 4 mV could be detected optically[13].

In the same experiment as that shown in Fig. 1, seven additional light-guide-photodiodes were positioned over the images of seven other neurones so that the activity of eight neurones was monitored simultaneously. In addition to the action potential in the impaled cell (7), there were spontaneous action potentials in two other neurones (1, and 4). We are confident that the absence of spikes in the remaining traces results from the absence of action potentials in those cells. (In several experiments, when a nerve root was stimulated with a suction electrode, optical spikes, presumably representing antidromic action potentials, appeared in previously silent neurones. These experiments are reported more fully in ref. 13.)

The magnitude of the optical signal will depend on several factors including membrane area and the amount and site(s) of dye binding in addition to the magnitude of the change in membrane potential. Since it is difficult to evaluate each of these parameters, it is not possible at present to determine the magnitude of the potential changes from these optical measurements; only information about the time course and the direction of the potential changes is obtained. Neither pharmacological effects nor photodynamic damage due to the merocyanine–oxazolone dye were detected in experiments on barnacle ganglia. Our evaluation of such effects is preliminary, however.

We think that the apparatus used in the experiment shown in Fig. 2 can be expanded so that 20 neurones are monitored simultaneously. It may be possible, using an electronic image detector positioned at the objective image plane, to develop an apparatus that monitors activity in 50–100 neurones. The ability to monitor activity from many cells may prove valuable in helping to determine the functional connections between neurones in nervous systems with a small number of neurones. Experiments designed to determine the neuronal basis of very simple behaviours might also be facilitated.

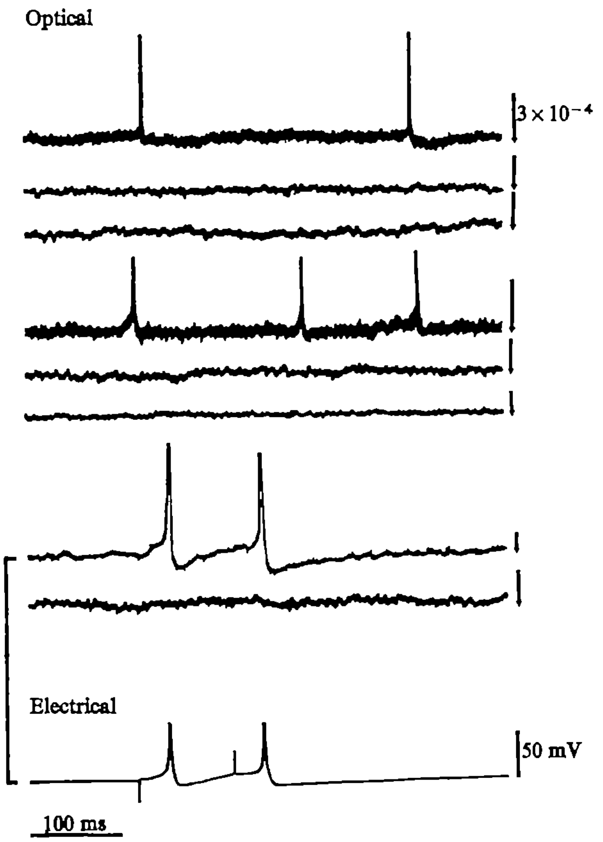

Fig. 2 Simultaneous optical recording from eight neurones. Neurone 7 was stimulated by passing current through the microelectrode; spontaneous action potentials are recorded from neurones 1 and 4. Each arrow represents a $\Delta A/A$ of 3×10^{-4}. ($\Delta I/I$, the observed fractional change in light intensity reaching the photodetector, is about half as much.)

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Antagonism of ethanol narcosis by histidyl-proline diketopiperazine

THYROTROPIN-RELEASING hormone, pyroglutamyl-histidyl-prolineamide (TRH, Fig. 1) belongs to the class of hypothalamic peptides termed releasing factors[1]. Although TRH was originally described as the hormone which stimulates the release of thyrotropin, it has subsequently been shown to be involved in many other biological processes[1]. The mechanism by which TRH exhibits this broad range of activities is not clear; it might involve the action of TRH at multiple recognition sites or TRH might serve as a precursor for compounds which are biologically active. Previous studies on TRH metabolism have demonstrated the formation of pyroglutamyl-histidyl-proline (acid TRH, Fig. 1)[1]. Attempts to associate biological activity with this compound have been uniformly negative, providing no support for the precursor hypothesis[2,3]. We show here that, in addition to acid TRH, TRH is also metabolised to histidyl-proline diketopiperazine (His-Pro, Fig. 1)[4,5]. This cyclic dipeptide is substantially more potent than TRH in reducing ethanol-induced sleep in rats[6]. These data indicate that TRH metabolism plays a part in its biological effects.

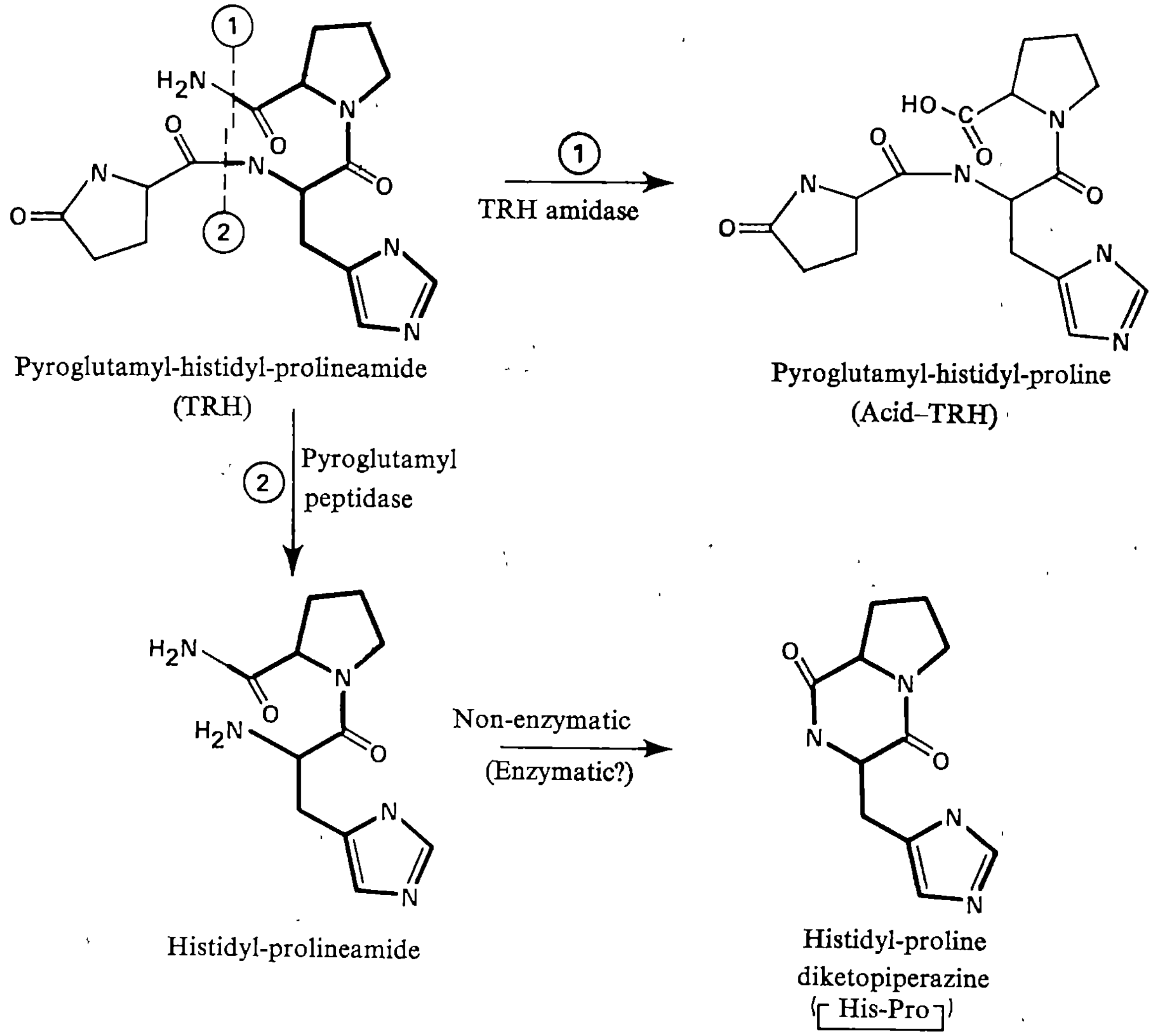

Fig. 1 Pathway of TRH metabolism in rat brain. The portion of the structure of TRH designated by the heavy lines corresponds to the region which gives rise to histidyl-prolineamide and histidyl-proline diketopiperazine. Synthetic L-histidyl-L-proline diketopiperazine was prepared by coupling benzyloxycarbonyl-L-histidine (20 mmol, ICN) with L-prolineamide (20 mmol, Vega-Fox) in the presence of hydroxybenzotriazole (40 mmol, Aldrich), *N*-ethylmorpholine (20 mmol, Aldrich) and dicyclohexylcarbodiimide (20 mmol, Pierce). The solution in 175 ml of dimethylformamide was stirred in an ice-bath for 3 h and then overnight at room temperature. After removal of the precipitated dicyclohexylurea, the solution was concentrated to dryness *in vacuo*, then dissolved in approximately 25 ml of H_2O. The solution was applied to a column (3 × 20 cm) of diethylaminoethylcellulose (Whatman DE-23, free base) previously equilibrated with H_2O and eluted with H_2O (10-ml fractions). Those fractions which were Pauly positive[10] were pooled and concentrated to dryness *in vacuo*. The intermediate benzyloxycarbonyl-L-histidyl-L-prolineamide was crystallised four times from hot H_2O (yield approximately 500 mg; microanalysis for C,H,N,O, (Microanalysis, Inc., Wilmington, Delaware), showed (in %): 58.94, 6.04, 18.04, 16.90). The calculated values are: 59.21, 6.02, 18.17, 16.60. Benzyloxycarbonyl-L-histidyl-L-prolineamide (500 mg) was dissolved in a solution containing methanol (10 ml), H_2O (0.3 ml) and glacial acetic acid (0.3 ml). After adding palladium black catalyst (100 mg), the suspension was hydrogenated under 40 pounds pressure for 2 h. The catalyst was removed by filtration and the resultant solution was heated in a boiling H_2O bath for 30 min, then dried *in vacuo* After dissolving the product in H_2O, it was purified by passage through a column (2 × 5 cm) of DEAE–cellulose; the column was washed with H_2O. The Pauly-positive fractions were concentrated to dryness *in vacuo* to yield crystalline L-histidyl-L-proline diketopiperazine (yield approximately 200 mg). Microanalysis for C,H,N,O showed (in %): 54.75, 6.08, 22.10, 17.22. The calculated values for His-Pro·0.6 H_2O are: 53.91, 6.25, 22.86, 16 97. Mass spectrographic analysis showed prominent ions at *m/e* 234 (the molecular ion of His-Pro), *m/e* 154 (the proline-glycine diketopiperazine ion), *m/e* 81 (the methyl imidazole ion) and *m/e* 70 (the pyrroline ion). ^{1}H-magnetic resonance spectral analysis at 100 MHz showed chemical shifts at 1.95, 2.2, 3.4, 3.5, 4.65, 7.4 and 8.25 p.p.m. downfield from trimethylsilane. Amino acid analysis of an acid hydrolysate (6 M HCl, 15 pounds inch^{-2}, 180 min) showed the presence of ninhydrin-positive material corresponding only to proline and histidine (ratio 1.1:1).

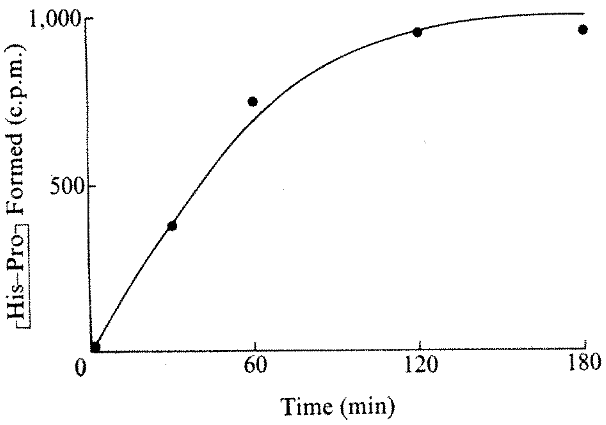

Fig. 2 Non-enzymatic formation of His-Pro from His-ProNH$_2$. [^{3}H-Pro]-His-ProNH$_2$ was prepared from [^{3}H-Pro]-TRH (New England Nuclear) as follows: 41 μmol of [^{3}H-Pro-]TRH (1.21 μCi μmol^{-1}) was incubated with 50 μmol Tris-HCl (*p*H 7.5) and 800 μl *Aerobacter cloacae* pGlu-peptidase (purified up to the Sephadex G-100 stage as described by Doolittle and Armentrout[7], activity = 0.3 μmol pGlu-Ala hydrolysed min^{-1} ml^{-1}) for 3 h at 25 °C in a total volume of 1 ml. At the end of the incubation period, the whole reaction mixture was applied to a sheet of Whatman 3 MM (60×45 cm) paper and chromatographed in 1-butanol–acetic acid–H$_2$O (4:1:1) (ascending, overnight, 25 °C). The area on the paper corresponding to His-ProNH$_2$ (R_f = 0.12–0.13, 4.7% of the total radioactivity) was cut out and extracted with 10 ml of 90% methanol. The methanol extract was filtered and concentrated *in vacuo* at 25 °C. The [^{3}H-Pro]-His-ProNH$_2$ comigrated with authentic His-ProNH$_2$ on paper chromatography (ascending, 25 °C, 15 h, Whatman 3 MM, chloroform–methanol–H$_2$O (5:5:1), (Solvent 1) R_f = 0.42) and paper electrophoresis (25 °C, Whatman 3 MM paper, 15 V cm^{-1}, 60 min, formic acid–acetic acid–H$_2$O (31:59:910), *p*H 2.0). [^{3}H-Pro]-His-ProNH$_2$ (1.9 μmol; 1.21 μCi μmol^{-1}) was incubated in PBS (200 μl) at 37 °C. At the times, indicated aliquots (20 μl) of the reaction mixture were spotted on a sheet of Whatman 3 MM paper. After overnight chromatography in solvent 1, the areas corresponding to His-Pro (R_f = 0.73) were cut out and counted in a liquid scintillation counter.

Our work has demonstrated the presence in extracts of hamster hypothalamus[4] and rat brain (unpublished observations) of the enzyme pyroglutamylpeptidase (L-pyroglutamyl peptide hydrolase, EC 3.4.11.8) which converts TRH to histidyl-prolineamide. This metabolite is unstable and readily cyclises to histidyl-proline diketopiperazine (His-Pro) (Fig. 1). The data in Fig. 2 document the instability of histidyl-prolineamide in phosphate buffered saline (PBS; 0.1 M potassium phosphate + 0.14 M NaCl, *p*H 7.2). ^{3}H-proline-histidyl-prolineamide was prepared by incubation of ^{3}H-proline-TRH with partially purified bacterial pyroglutamyl-peptidase[7]. Incubation of ^{3}H-proline-histidyl-prolineamide at 37 °C in PBS resulted in rapid cyclisation to the diketopiperazine. The half life of histidyl-prolineamide in these conditions was approximately 40 min. These data suggest that the pyroglutamylpeptidase-dependent conversion of TRH in brain to histidyl-prolineamide is probably followed by a rapid cyclisation to His-Pro.

The results of a further study (Fig. 3) provided additional evidence that His-Pro is an *in vivo* metabolite of TRH. [^{3}H-Proline]-TRH (20 μl) was injected into the right lateral ventricle of a rat[8]. The animal was decapitated 5 min after the injection; the brain was then removed and extracted with 10 ml of 0.4 N HClO$_4$. Paper chromatography of the neutralised HClO$_4$ extract (see legend Fig. 3) revealed that, in addition to residual TRH, radioactivity was localised in acid TRH,, linear histidyl-proline and His-Pro (approximately 8% of the total radioactivity). Incubation of acid-inactivated brain with radioactive TRH, followed by the same extraction procedure, indicated that TRH was not degraded non-enzymatically. The linear histidyl-proline was probably either derived from acid TRH by the action of pyroglutamylpeptidase or from histidyl-prolineamide by the action of TRH amidase[4]. The scheme shown in Fig. 1 summarises our present theory on the pathway for TRH metabolism in brain. We have established the existence of enzymes forming acid TRH and histidyl-prolineamide[4]. Although the existence of an enzyme catalysing the conversion of histidyl-prolineamide to His-Pro seems likely, the instability of histidyl-prolineamide has, thus far, hindered out attempts to detect such an activity.

Breese *et al.*[6,9] reported that TRH antagonises the effect of ethanol in inducing sleep in mice and rats; acid TRH did not substitute for TRH in this reduction of ethanol narcosis. Our findings that His-Pro is another metabolic of TRH prompted us to test its effect on ethanol narcosis. Figure 4 shows concentration curves for effectiveness of TRH and His-Pro in reducing ethanol-induced sleep. The data confirm the finding[6,9] that TRH antagonises ethanol narcosis and that acid TRH is inactive. There was no effect on the time for loss of the righting reflex, but TRH significantly decreased the righting time. The more important finding, however, is that His-Pro is substantially more effective than TRH in this test. The data suggest that a reduction of sleep time to 50% of the control requires intraventricular injection of 1 μmol kg^{-1} of His-Pro or 50 μmol kg^{-1} of TRH The study shown in Fig. 3 indicated that the intraventricular injection of 1 μmol kg^{-1} of TRH leads to the formation of approximately 0.08 μmol kg^{-1} of His-Pro. Figure 4 shows that 1 μmol kg^{-1} of TRH or 0.08 μmol kg^{-1} of His-Pro produce comparable reductions in ethanol-induced sleep. This observation is compatible with the notion that the activity of TRH in antagonising ethanol narcosis requires its conversion to His-Pro.

We previously introduced the hypothesis that the metabolism of TRH may generate compounds with biological

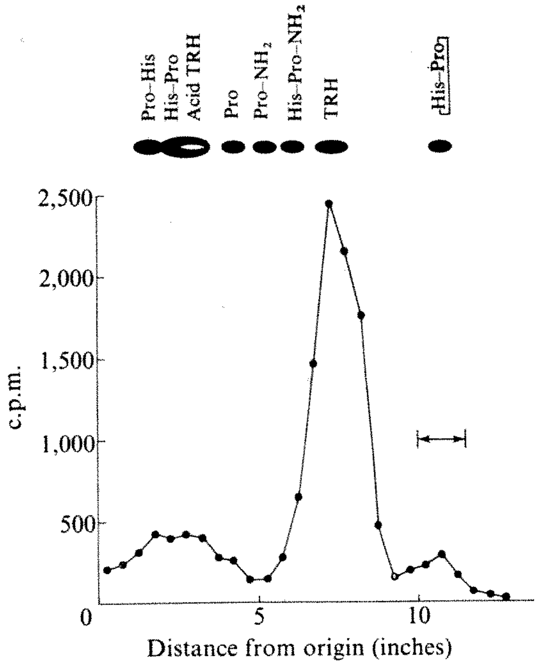

Fig. 3 Formation of His-Pro from intraventricularly administered TRH. A male Sprague-Dawley rat (approximately 100 g) was injected with 20 μl [^{3}H-Pro]-TRH (10 μc, 100 μCi μmol^{-1}) in saline. The animal was decapitated 5 min after injection, and the brain quickly removed. The brain was homogenised in 0.4 N HClO$_4$ (10 ml) and the centrifuged extract adjusted to *p*H 6.4–6.6 with saturated KHCO$_3$ solution. The supernatant solution was lyophilised and then extracted with a small volume of 90% methanol. The methanol extract was applied in a 30-cm band to a sheet of Whatman 3 MM paper and chromatographed (ascending) in solvent 1. The chromatogram was dried and a 1-inch strip was cut into 0.5 inch segments and counted. The area corresponding to His-Pro (10–11.5 inches from the origin) was extracted with methanol and rechromatographed (ascending) in chloroform–methanol–NH$_4$OH (60:45:20). The radioactivity comigrated with authentic His-Pro (R_f = 0.73).

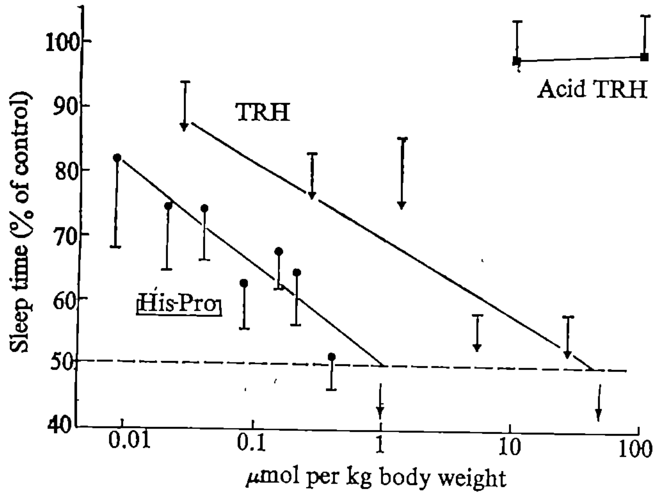

Fig. 4 Effect of TRH and His-Pro on sleep induced by ethanol. All animals were injected intraperitoneally with 8 ml ethanol (50% v/v in saline) per kg body weight. TRH, acid TRH or His-Pro (dissolved in saline) were administered to rats intraventricularly (15–18 μl per animal) under light ether anaesthesia 10 min before the ethanol injection. The concentrations of the peptide solutions were such that 100 μl contained the indicated number of μmol. Sleeping time for animals was determined as described earlier[6]. The sleep time for control animals receiving saline intraventricularly and ethanol intraperitoneally was 69±8 min ($n = 17$). Each point represents 6–10 animals (150–180 g) and the data are presented as mean±s.e.m. The horizontal dashed line corresponds to a 50% decrease in the sleep time. The arrows point to the concentrations of His-Pro and TRH required to effect a 50% decrease in the sleep time induced by ethanol.

activity[4] and the data presented here validate this idea. It will be of interest to see whether His-Pro corresponds to the 'active' form of TRH in a number of other systems where TRH has shown effects. It seems unlikely that TRH metabolism is obligatory for all of its effects since neither acid TRH nor His-Pro can replace TRH in reducing pentobarbital-induced sleep (preliminary experiments). Thus, the complete role of His-Pro in 'TRH-mediated' functions remains to be established.

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Eighty thousand β-adrenoreceptors in a single cell

CATECHOLAMINES function as hormones in peripheral organs and as neurotransmitters in the central nervous system (CNS). The primary event in the action of catecholamines is their interaction with specific α-adrenoreceptors or β-adrenoreceptors[1]. The interaction of catecholamines with β-adrenoreceptors induces the activation of the enzyme adenylate cyclase which converts adenosine triphosphate (ATP) to cyclic AMP (ref. 2). Development of reliable radioligand binding methods has enabled the direct determination of the number of β-adrenoreceptors in various cell types[3–13]. The content of β-adrenoreceptors in all the cells examined varies between a few hundred and a few thousand receptors per cell. These values correspond to $(0.2–2.0)\times10^{-12}$ mol receptor per mg membrane protein, when membranes are prepared from these cells.

The absence of a rich source for β-adrenoreceptors had hindered attempts to identify and purify the receptor molecule. A report by Schubert *et al.*[13] showed that skeletal muscle myoblasts (L6 cells) grown in culture respond to (−) noradrenaline by a 500-fold increase in their intracellular content of cyclic AMP and this finding prompted us to examine the β-adrenoreceptor density of these cells. We report here on the presence of 80,000 β-adrenoreceptors per cell in L6P cells grown in culture, as determined by a direct binding of ^{125}I-(±)-iodohydroxybenzylpindolol (^{125}I-(±)-HYP) to intact cells. In parallel, we examined the direct binding method by measuring ^{125}I-(±)-HYP binding to intact turkey erythrocytes.

Rat skeletal myoblasts polyploid cells[14] (L6P) were obtained from Dr D. Yaffe. The cells grown on collagen were detached from their support by incubation with ethylene diamine tetraacetate (EDTA) 1.5 mM for 2 h, washed and suspended in the isotonic buffer and ^{125}I-(±)-HYP binding was measured. The experimental details are given in Fig. 1. The binding of ^{125}I-(±)-HYP to intact turkey erythrocytes was also measured (Fig. 2) using similar conditions. Both L6P cells and turkey erythrocytes demonstrate a single class of ^{125}I-(±)-HYP binding sites (Figs 1*c* and 2*c*) and exhibit the same affinity towards the radiolabelled β-blocker (Figs 1 and 2 and Table 1).

L6P cells were found to possess 80,000 ^{125}I-(±)-HYP

Table 1 Number of β-adrenoreceptors in intact L6P cells and intact turkey erythrocytes

Cell type	Number of ^{125}I-(±)-HYP binding sites per cell	^{125}I-(±)-HYP receptor dissociation constant (M)	Ref.
L6P	80,000	1.74×10^{-10}	This study
Turkey erythrocytes*	1,100	1.60×10^{-10}	This study
Turkey erythrocytes	400–500	1.9×10^{-11}	12

* Turkey erythrocyte membranes were found to have 0.9 pmol receptor per mg membrane protein, using ^{3}H-(±)-propranolol binding[3, 4]. On the basis of this finding it was calculated that an intact turkey erythrocyte has 550±120 receptors per cell[3]. Using equilibrium dialysis with ^{3}H-(±)-isoproterenol, the number of specific β-adrenoreceptors was found to be 1,200±300 receptors per cell[3].

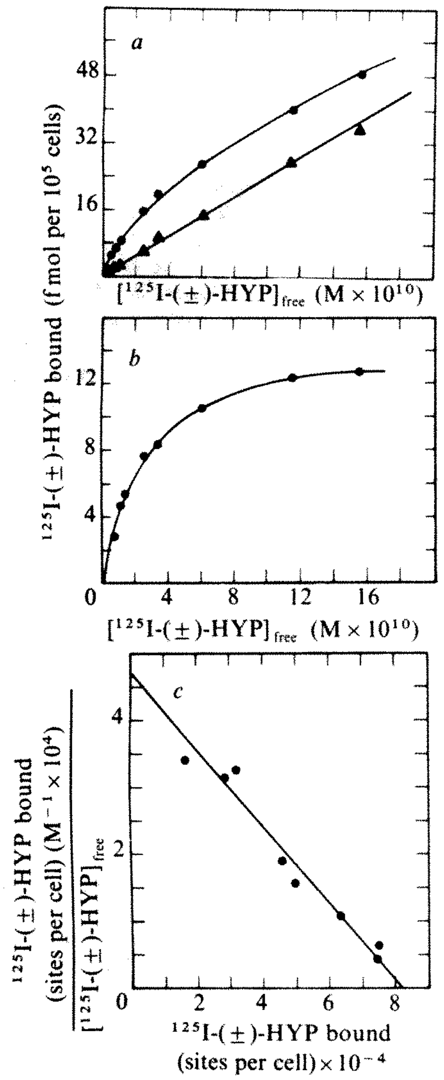

Fig. 1 Binding of ^{125}I-(±)-HYP to intact L6P cells. ^{125}I-(±)-HYP was prepared and purified as described previously[9] and L6P skeletal muscle myoblasts were grown in plastic bottles for 9 d as described[14]. To detach the cells, the growth medium was removed and the clone incubated with 10 mM Tris-HCl *p*H 7.4 containing 1.5 mM Na_2EDTA and 140 mM NaCl at 37 °C for 2 h. The surface was then scraped gently with a rubber stick to detach the cells from the surface. On exposure to the detaching buffer, the cells became round within 15 min as viewed under the microscope. The suspended cells were then centrifuged for 5 min at 950*g* and the washed cells were resuspended in 1.2 ml of the above buffer, counted and used for the ^{125}I-(±)-HYP binding assay. Fifty microlitres containing 1.0×10^5 cells ml^{-1} or 1.0×10^6 cells ml^{-1} were mixed with 0.45 ml of the buffer which contained increasing concentrations of ^{125}I-(±)-HYP ($0.2–15.6 \times 10^{-10}$ M) in the absence and in the presence of DL-propranolol (5.0×10^{-5} M final concentration). Thus the final concentration of cells in the binding mixture was 1.0×10^4 cells ml^{-1} in one set and 1.0×10^5 cells ml^{-1} in the second set. The binding mixtures were incubated for 30 min at 37 °C. Subsequently 0.45 ml of each binding mixture was filtered on a Whatman GF/C glass filter as previously described[9, 12] and counted in a gamma counter (Packard). A sample of 50 µl from each binding mixture was counted to determine the total ^{125}I-(±)-HYP concentration. From the values obtained for the bound ^{125}I-(±)-HYP and the total ^{125}I-(±)-HYP concentration in the binding mixture, the values of [^{125}I-HYP]$_{free}$ were computed. Each point represents the mean of two determinations. The data presented are for 1.0×10^5 cells ml^{-1} final concentration in the binding assay. *a*, Total ^{125}I-(±)-HYP bound; ▲, ^{125}I-(±)-HYP bound in the presence of 5.0×10^{-5} DL-propranolol (nonspecific binding). *b*, Specific ^{125}I-(±)-HYP bound. *c*, Scatchard plot of data in *b*. The data in *b* were converted from fmol per 10^5 cells to number of receptors per cell. Maximal binding was found to be 13.4 fmol ^{125}I-(±)-HYP per 10^5 cells ($13.4 \times 6.03 \times 10^{23} \times 10^{-15}$ ^{125}I-(±)-HYP sites per 10^5 cells, which equals 80,400 receptors per cell; 6.03×10^{23} is Avogadro's number). Analysis of binding using 10^4 cells ml^{-1} yields 81,200 receptors per cell.

binding sites (Fig. 1), whereas turkey erythrocytes were found to possess 1,100 (Fig. 2). The latter value is close to the number of β-adrenoreceptors calculated on the basis of ^{3}H-(±)-propranolol binding to turkey erythrocyte membranes and to the value reported by Brown *et al.*[12], who measured ^{125}I-(±)-HYP binding to intact turkey erythrocytes (Table 1). The binding of ^{125}I-(±)-HYP in both cell types is inhibited by (±)-propranolol (Figs 1 and 2), a specific β-adrenergic blocker, and is completed within 20 min of incubation. The maximal capacity of ^{125}I-(±)-HYP binding was found to be directly proportional to the cell concentration in the range of 10^4 cells ml^{-1} to 5×10^5 cells ml^{-1} for L6P cells, and in the range 1.0×10^6 to 5.0×10^7 cells ml^{-1} for turkey erythrocytes, confirming that equilibrium was reached between the cells and the radiolabelled ligand within the incubation time used.

In conclusion, it seems that the L6P cell line can become

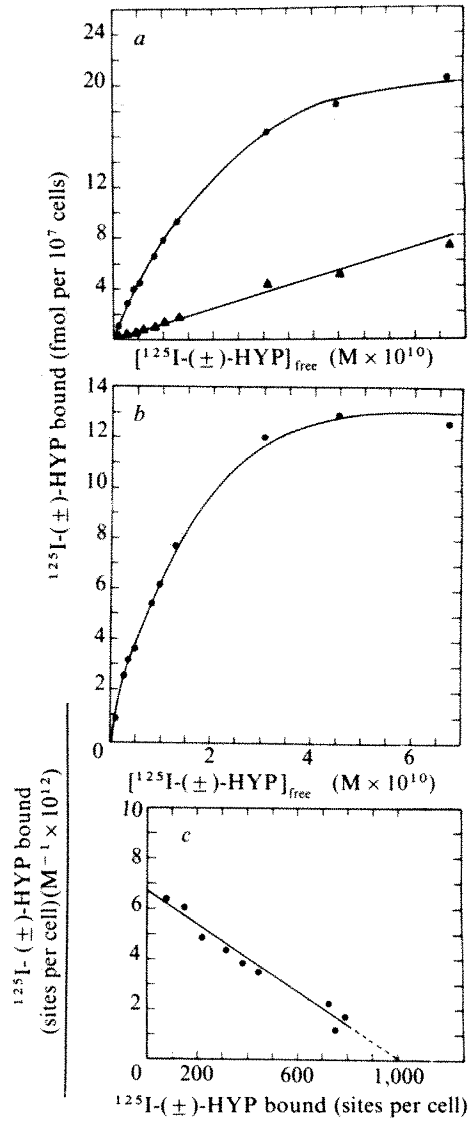

Fig. 2 Binding of ^{125}I-(±)-HYP to intact turkey erythrocytes. The preparation of a cell suspension of turkey erythrocytes essentially followed a published procedure[12, 15]. Fresh heparinised blood from adult turkeys was centrifuged at 400*g* for 10 min at 4 °C. This step was repeated four times. The washed erythrocytes were resuspended in 10 mM Tris-HCl *p*H 7.4 containing 0.14 M NaCl, 10 mM KCl, 11 mM glucose and 50 units ml^{-1} penicillin. A final concentration of 1.0×10^6 cells ml^{-1} and of 1.0×10^7 cells ml^{-1} were used for the binding experiments. The following steps were identical to those described in the legend to Figure 1. *a*, Total ^{125}I-(±)-HYP bound; ▲, ^{125}I-(±)-HYP bound in the presence of 5.0×10^{-5} M (±)-propranolol. *b*, Specific ^{125}I-(±)-HYP bound. *c*, Scatchard plot of data in *b*.

a good candidate for a rich source of β-adrenoreceptors. The access to such a source may advance the efforts aimed at the chemical characterisation of the β-adrenoreceptor and possibly its purification.

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How motoneurones control development of muscle tension

THE sum of all synaptic currents flowing at any moment through the motoneurone membrane represents the synthesis of the commands converging on to the final common path. Transduction of such commands into muscle contraction is accomplished by the conversion of current into motoneuronal firing and, in turn, conversion of the nervous discharge into mechanical events[1]. Thus, the motor unit, that is, each motoneurone with the muscle fibres it innervates, operates as the interface between the central nervous system and the environment. The motor unit response to steady inputs is well known. Constant currents are transformed into constant-rate discharges, whose frequencies are proportional, over a definite range, to the current intensities[2]; in isometric conditions, such constant-rate discharges build up steady and proportional levels of muscle tension[3-6]. On the other hand, little is known about the motor unit response to dynamic inputs. Recent work[5], however, has suggested that the rate of growth of the current stimulating the motoneurone may be converted into a proportional rate of development of the muscle tension. In view of this last hypothesis, we have analysed the isometric tension developed by single motor units when motoneurones were stimulated with current pulses of trapezoidal shape, that is, reaching a given steady level after rectilineal (ramp) growth of different slopes. This approach was adopted on the assumption that current injected by an intracellular electrode into a motoneurone is physically equivalent of total synaptic current acting on the cell[1,2].

In cats the spinal cord was transected at high cervical level under sodium pentobarbital anaesthesia (40 mg per kg body weight), the lumbar cord was exposed and the tendon of the gastrocnemius-soleus (G–S) muscle cut and attached to an isometric force transducer (Grass FT03). The muscle was stretched and maintained at the length giving the maximal isometric twitch response. The activity of G–S motoneurones was recorded intracellularly using glass microelectrodes (3 M KCl) through which depolarising current pulses of trapezoidal shape (total duration 200–250 ms) were passed across the cell membrane. The rising phase of the pulses was rectilineal and the rise-time variable between 0 (rectangular pulse) and 120 ms. In some experiments, the trapezoidal currents were superimposed on a current step of rheobasic intensity. This experimental procedure has been successfully completed in nine motor units, all belonging to the fast type[6] (time-to-peak of the single twitch: 18–25 ms). In eight of the nine the trials could be repeated for several different values of the steady-state current. Shape of the current pulses, motoneuronal discharge and isometric tension developed by the motor units were simultaneously displayed on the oscilloscope and photographed.

Constant-rate firing of a motoneurone can be obtained by two different methods, each leading to a different mode of muscle tension development. The motoneuronal discharge may be obtained by a train of individual short current pulses, each eliciting a single spike (Fig. 1*a*). Alternatively, rhythmic firing can be evoked by a long-lasting rectangular current step (Fig. 1*b*): in this case a constant-rate discharge of the same frequency as in Fig. 1*a* is reached through adaptation, that is, after the fall of the high instantaneous frequency of the first interspike interval. Such a frequency reduction is caused by summation of the conductance changes underlying the spike's afterhyperpolarisation (AHP) (refs 7–11). The related tension recordings show that muscular response is much faster and stronger in (*b*) than in (*a*). In (*b*) firing attains the same frequency as in (*a*) just after the second spike: the difference in tension seems therefore to be attributable to the initial, non-adapted, high-frequency discharge produced by the rectangular current. This suggests that modulation of the high-frequency, non-

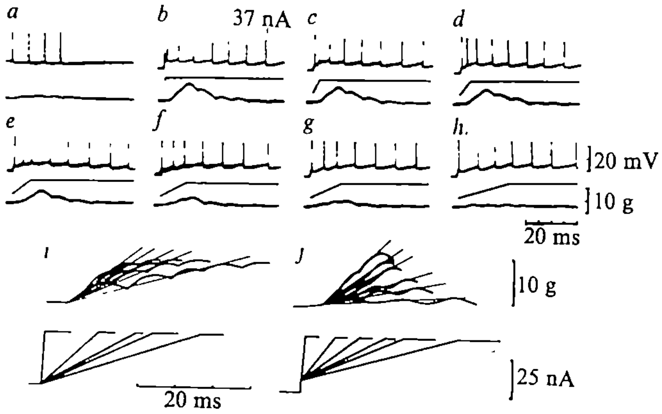

Fig. 1 Response of a motor unit to rectilineal input transients of different slopes. *b–h*, G–S motor unit of the fast type (time to peak for the single twitch 20 ms). For each recording, the middle trace represents the time-course of the motoneurone-stimulating current, the uppermost the neuronal discharge, the lowermost the mechanical response. Decrease in the slope of the current rising phase is accompanied by a parallel decrease in the peak initial tension and in the velocity of tension development. This is mediated by a rearrangement of the early phase of motoneurone firing, whose major feature is a progressive lengthening of the first interspike interval. With the slowest-rising ramp (*h*), firing frequency is nearly regular from the very beginning. Profile of isometric tension is, in this trial, similar to that obtained by single-pulse, constant-rate stimulation of motor cell (*a*). *i*, *j*, Upper traces are superimposed drawings of the tension profiles recorded in the same unit (*i*) and in another (*j*) fast G–S unit (single twitch time-to peak 25 ms) in which the amplitude of peak initial tension was not affected by the slope of stimulating current (lower traces). Straight lines connect the onset of tension with the points where 90% of peak tension is reached in each trial.

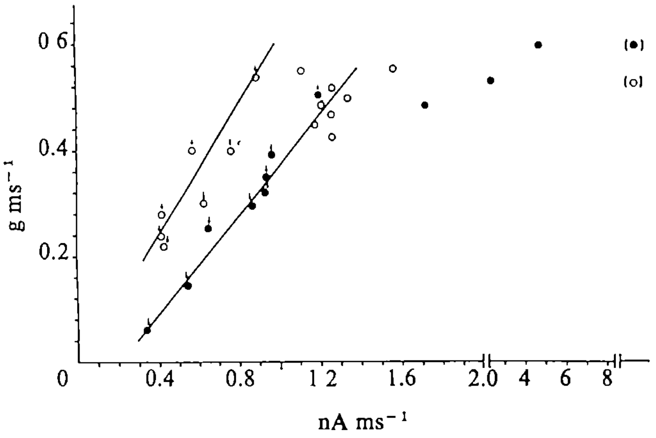

Fig. 2 Correlations between velocity of current commands to motoneurones and velocity of muscle tension recruitment Same units as in Fig. 1*j* (○) and *i* (●). Abscissa: velocity of the rising phase of the currents injected into the motoneurones in nA ms^{-1}. Ordinate: velocity of development of isometric tension, calculated as the slope of the straight lines drawn in Fig. 1 *i* and *j*. Points within brackets (on the right) signal the tension velocity developed by a rectangular pulse. Straight lines represent the regression functions calculated by the least squares method from the points marked by arrows. Note scale changes on abscissa.

adapted discharge may lead to a correlated modulation of the initial, transient phase of the tension profile.

The same motor unit was then stimulated with trapezoidal current pulses reaching the same level as in Fig. 1*b*, but after ramps of different slopes. Figure 1 *c–h* shows that decreasing the ramp slope has a clear-cut effect on the initial part of the motoneuronal discharge but leaves steady-state firing untouched. The effect consists of a progressive increase in duration of the first interspike interval while the second and eventually (Fig. 1*h*) the third intervals undergo more complex changes. The relationships between changes in the ramp slope and rearrangement of the motoneuronal initial discharge will be analysed in detail elsewhere. It is of value here to follow the manner in which such slope changes modify the tension profiles. Figure 1 *c–h* shows that a slowing down of the current rising phase is paralleled by a proportional reduction of both the absolute value of muscle tension and of its slope. This is more apparent in Fig. 1*j*, where the tension profiles from six records are superimposed.

Velocity of tension development was estimated from the experimental records by calculating the slope of the straight lines connecting the tension onset to the point at which 90% of maximal force was attained in each trial (Fig. 1*j*). This velocity has been plotted against the rise velocity of the related currents, as illustrated by the filled circles in Fig. 2. It is evident here that the two velocities are directly related to each other. Correlation seems to be linear over a considerable range of values, from about 0.3 nA ms^{-1} up to 1.2 nA ms^{-1}. Ramps steeper than 1.2 nA ms^{-1} do not increase the velocity of tension development much further (maximal velocity, attained with the rectangular current step, is represented by the dot within brackets). Such an insensitivity to the highest ramp slopes can be directly appreciated by comparing records (*c*) and (*b*) in Fig. 1.

In this motor unit, as well as in four other units in the present material (see also refs 5 and 12), tension responses to fast transients display an initial peak whose amplitude is positively correlated with ramp velocity (Fig. 1 *c–h*). Such a peak might be important in non-isometric conditions, when an initial extra force may be required for quickly overcoming inertia at the start of the movement. In these units, the behaviour described above was less evident, or even disappeared, when the strength of the current pulse was increased: this produced a proportional increase of steady-state firing rate, which eventually became sufficiently high to keep the tension at (or close to) the level reached initially. Figure 1*i* refers to the response encountered in the remaining four units and represents a series of tension profiles obtained from one of them and superimposed as in Fig. 1*j*. These profiles do not show any initial peak: whatever the strength of the current pulse, motoneuronal firing frequency at steady state (not illustrated) was always high enough to prevent the tension from falling after adaptation. In this unit, variations of the ramp slope are accompanied only by proportional changes in the slope of tension development. Velocity plot (Fig. 2, open circles) displays the same type of correlation described in the preceding example. This correlation was also consistently found in all other units investigated, independently of the presence or absence of the initial tension peak.

The linear transduction of the velocity of transients demonstrated here seems to depend on special properties of both components of the motor unit. Motoneurones encode velocity of the rising phase of a trapezoidal input signal by modulating the frequency of their initial, non-adapted discharge. The firing response to a current step represents the borderline example of such a modulation. It is accepted that the changes in firing frequency (that is, adaptation) observable in that condition are caused by the kinetics of summation of the AHP conductance changes[10,13,14]. It therefore seems justifiable to assume that the same mechanism is also responsible for the modulation of motoneuronal discharge in response to ramps of different slopes.

At the muscular level, a feature which should prominently contribute to regulation of the rate of tension recruitment are the so called 'catch properties'[15], that is, the strong 'potentiation' of the muscle twitch following the second of two short-interval stimuli[15,16].

It can be concluded that the motor unit is provided with a mechanism for independent transduction of both the steady levels and the transients of the input signals. While the steady level of isometric tension is proportional to the steady intensity of the current command to the motoneurone, velocity of force development can be regulated (over a definite range limited by the intrinsic muscle properties) by the command slope.

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Acetylcholine-like effects of intracellular calcium application in pancreatic acinar cells

INDIRECT evidence suggests that Ca^{2+} is the mediator of the effects of acetylcholine and cholecystokinin-pancreozymin in pancreatic acinar cells. Sustained stimulation-evoked acinar fluid and enzyme secretion depends on extracellular Ca^{2+} (refs 1,2), stimulation causes uptake of ^{45}Ca^{2+} from the extracellular fluid[3] as well as release of ^{45}Ca^{2+} from prelabelled glands with a dose–response curve very similar to stimulation-evoked membrane depolarisation and enzyme secretion[4] and the Ca^{2+} ionophore A23187 induces enzyme secretion[5] and membrane depolarisation[6]. We have examined directly the bioelectrical effects of iontophoretic application of Ca^{2+} from intracellular microelectrodes in a mammalian gland cell preparation.

Two separate microelectrodes, one filled with either 0.2 M CaCl$_2$ (or sometimes 0.2 M MgCl$_2$) plus 0.05 M EGTA (ethylene glycol-*bis*-β (amino-ethylether)-*N*,*N'*-tetraacetic acid) (*p*H 7) or 3 M KCl and the other always filled with 3M KCl, were inserted very close to each other (20–50 μm intertip distance) into communicating surface acinar cells of mouse pancreas superfused *in vitro*. The tip of a third microelectrode, filled with 2M AChCl, was placed close to the impaled acinus in an extracellular position. The magnitude of iontophoretic currents was measured using a virtual ground current–voltage converter. Apart from one of the intracellular electrodes most often containing CaCl$_2$, the set-up was identical to that used to determine the ACh null potential in pancreatic acinar cells[7]. The composition of the physiological saline solution flowing through the tissue bath was (mM): NaCl, 103; KCl, 4.7; CaCl$_2$, 2.56; MgCl$_2$, 1.13; NaHCO$_3$, 25; NaH$_2$PO$_4$, 1.15; D-glucose, 2.8; Na pyruvate, 4.9; Na glutamate, 4.9; Na fumarate, 2.7. The solution was gassed with 95% O$_2$, 5% CO$_2$.

Figure 1 shows the effects of extracellular ACh and intracellular Ca^{2+} applications in the same acinus. The effects of Ca^{2+} closely mimicked those of ACh causing membrane depolarisation, reduction of input resistance and reduction of membrane time constant. Such ACh-like effects of intracellular Ca^{2+} applications (2–20 nA, 500 ms depolarising pulses) were repeatedly obtained in 14 cells from eight glands. Hyperpolarising current pulses of this magnitude had no effect.

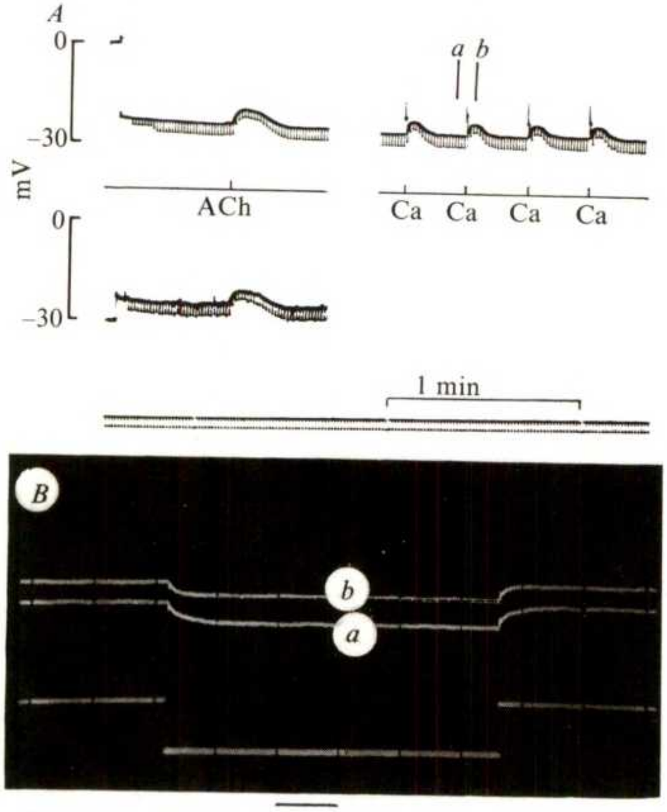


Fig. 1 The action of extracellular ACh and intracellular Ca^{2+} on membrane potential and resistance. *A*, The left-hand side of the trace shows the effect of a short pulse of iontophoretic ACh application (6×10^{-8} A, 0.5 s, retaining current 2×10^{-8} A) on membrane potential and resistance in two electrically coupled acinar cells. The upper trace represents the potential measured by an intracellular KCl electrode through which hyperpolarising rectangular current pulses (2×10^{-9} A, 100 ms duration) were injected repeatedly. The lower trace represents the membrane potential of a neighbouring cell recorded with a CaCl$_2$ electrode. At the point of interruption of the tracings, the CaCl$_2$ electrode was disconnected from the recording system and connected to a stimulator so that current could be injected through the electrode. At the marker signals labelled Ca, 5 nA, 0.5-s depolarising current pulses were applied through the Ca^{2+} electrode. *B*, The time course of membrane polarisation caused by injecting the 100-ms current pulse through the KCl electrode before and after the intracellular Ca^{2+} application (calibration: horizontal 20 ms, vertical 10 mV and 2×10^{-9} A).

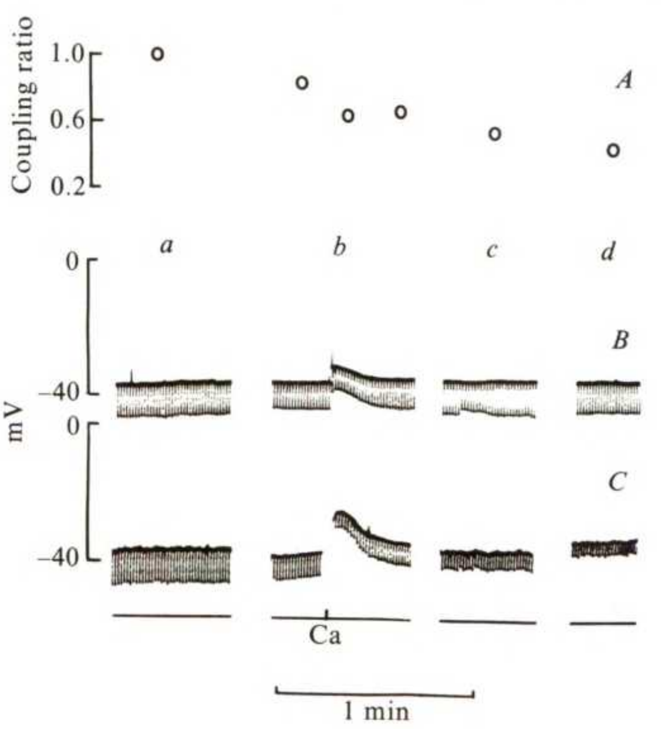
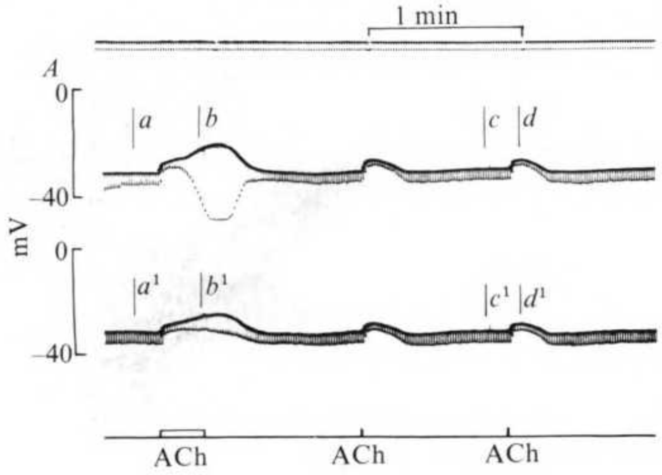


Fig. 2 The effect of repeated iontophoretic Ca^{2+} injections on acinar cell-to-cell coupling, membrane potential and resistance. *a–d*, Consecutive traces obtained from the same acinus with two indwelling electrodes in neighbouring communicating cells. *C*, Membrane potential measured by the CaCl$_2$-filled microelectrode. Iontophoretic ejections of Ca^{2+} (20 nA, 500 ms duration) were repeatedly carried out (for example section *b*). Between *a* and *b* (2.5 min interval) three such Ca^{2+} ejections occurred, between *b* and *c* (1 min interval) two ejections took place and between *c* and *d* (1.5 min interval) one ejection was made. *B*, Membrane potential measured by the KCl microelectrode. Through this electrode, current pulses (hyperpolarising, 2.5 nA, 100 ms duration) were passed repetitively. *A*, Coupling ratios (magnitude of electrotonic potential change in cell impaled by CaCl$_2$ electrode, caused by the hyperpolarising pulses injected through the KCl electrode, divided by the magnitude of the current pulse induced potential change in the cell impaled by the KCl electrode) corresponding to the situations in *a–d*.

Control experiments were carried out to exclude the possibility that these effects were unrelated to the intracellular Ca^{2+} application. The effects were not caused by the current passage or the potential change, since 2–20 nA depolarising or hyperpolarising current pulses applied through a KCl electrode never had any effect (six glands). Considerably larger current pulses (50–100 nA), however, caused membrane depolarisation and resistance reduction. These effects were observed after both depolarising and hyperpolarising current pulses, but the hyperpolarising pulses were by far the more effective. The shape of these potential changes was very different from those caused by Ca^{2+} injections. The depolarising phase was much steeper and the depolarisations were more short-lived, except in the case of the largest currents (> 100 nA). This phenomenon is probably caused by a reversible dielectric breakdown in the cell membrane (refs 8 and 9 and refs therein). The effects of Ca^{2+} injections were not evoked by releasing ACh from postsynaptic nerve endings surrounding the acini, since Ca^{2+} ejections with the CaCl$_2$ electrode extracellularly had no effect, probably reflecting the paucity of 'close' innervation[10] and since the effects of intracellular Ca^{2+} applications were also observed during exposure of the gland cells to 1.4×10^{-6} M atropine (three glands) a concentration abolishing the effects of extracellular



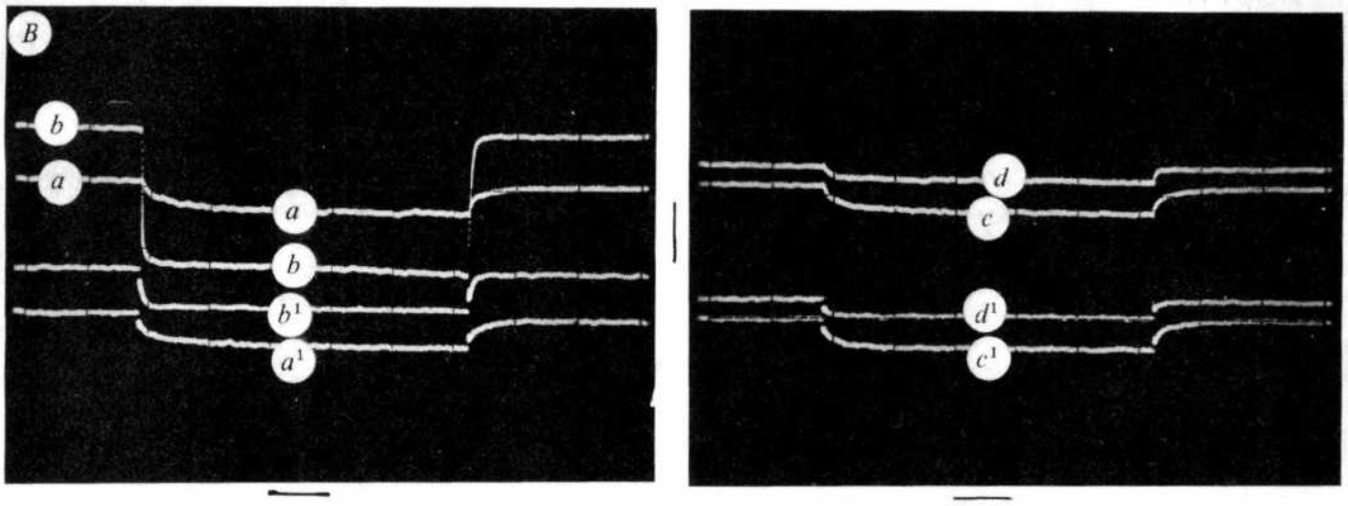


Fig. 3 The action of short and long pulses of ACh on membrane potential, resistance and electrical coupling. *A*, Two tracings of membrane potentials from two neighbouring acinar cells, both recorded with KCl electrodes. Hyperpolarising rectangular current pulses (2 × 10^{-9} A, 100 ms) were repetitively injected through the electrode recording the potential represented by the upper trace. The event marker signals represent the duration of the ejecting ACh pulses from the third extracellular microelectrode (6 × 10^{-8} A, retaining current 2 × 10^{-8} A). *B*, The shape and time course of the membrane polarisations in both cells caused by the current injections (calibration: horizontal 20 ms, vertical 10 mV).

iontophoretic ACh stimulation. The results of Ca^{2+} injections were not unspecific divalent cation effects since 2–20 nA, 500 ms depolarising current pulses applied through MgCl$_2$-filled electrodes (four glands) never caused any changes. Dielectric breakdown was observed after large current pulses (50–100 nA, both depolarising and hyperpolarising).

It is well known that intracellular Ca^{2+} injections can cause uncoupling of epithelial cells which are normally fully electrically coupled[11]. Small short-lived Ca^{2+} pulses resulting in the potential and resistance changes seen in Fig. 1 did not cause uncoupling. It was not possible to use much larger pulses since these evoked dielectric breakdown. After 5–10 min of repetitive Ca^{2+} injections in six glands, however, when it was still possible to get reliable recordings from the CaCl$_2$ electrode, uncoupling (that is, a reduction in the magnitude of electrotonic potential changes picked up by the CaCl$_2$ electrode caused by current injection through the KCl electrode in the neighbouring cell) was observed (Fig. 2). Uncoupling was never observed after Mg^{2+} or K$^+$ ejections.

The demonstration that intracellular Ca^{2+} applications result in membrane depolarisations similar to those evoked by ACh suggests, but does not prove, that the ACh effects are caused by a rise in cytosol Ca^{2+} concentration. Unfortunately the small size of pancreatic acinar cells (10–15 μm in diameter) has prevented the use of photoproteins (such as aequorin) to monitor changes in intracellular Ca^{2+} concentration following cell activation[8,11,12]. It does seem, however, that uncoupling is always caused by a rise in ionised Ca^{2+} concentration[11]. It is therefore of interest that prolonged extracellular ACh application can evoke reversible acinar cell uncoupling (Fig 3). It can also be seen in Fig. 3 that at the stage of ACh-evoked uncoupling, the depolarisation in one cell (the cell into which current pulses are injected) becomes more marked than in the neighbouring cell. When the cells subsequently recouple the effects of short doses of ACh become equal. Such ACh-evoked acinar cell uncouplings were observed repeatedly in four different glands. This phenomenon, which had been postulated on the basis of indirect evidence[13], is probably the most direct demonstration of an ACh-evoked increase in pancreatic acinar cytosol Ca^{2+} concentration available at present.

The new experimental findings that ACh can cause acinar cell uncoupling and that intracellular Ca^{2+} applications can mimic the effect of ACh provide new evidence for the hypothesis that ACh acts primarily on membrane Ca^{2+} translocation[14].

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Nuclear localisation of an oocyte component required for the stability of injected DNA

MOST cells contain deoxyribonucleases but do not degrade their nuclear DNA. This paradox is sometimes explained by assuming that the nucleases do not have access to DNA, from which they may be separated by the nuclear membrane. Another possibility is that cells contain a component which stabilises DNA even in the presence of nucleases. We show here that such a component is present in the frog oocyte, a type of cell in which deoxyribonucleases have been repeatedly demonstrated[1,2]. Although we have not characterised this component, we show that it is localised in the nucleus; it seems to be absent from cytoplasm but may leak into it when the nuclear membrane is ruptured. The DNA whose stability we have investigated is a small circular molecule introduced into the oocyte by microinjection, but the conclusions probably apply also to the endogenous nuclear DNA of the oocyte, and perhaps to the nuclear DNA of eukaryotic cells in general.

We performed three types of experiment in which *Xenopus laevis* oocytes were injected with purified ^{3}H-labelled SV40 DNA and then analysed singly by methods which show whether the injected DNA lay within the nucleus or in cytoplasm. First, using autoradiography, we defined the location of ^{3}H-SV40 DNA immediately after injections aimed at the oocyte nucleus (germinal vesicle, GV). Second, individual nuclei and cytoplasms, manually separated, were analysed for their content of ^{3}H-SV40 DNA several hours after injections aimed at either the GV or vegetal pole cytoplasm. Third, the molecular configuration of this recovered DNA was correlated with its location in each oocyte.

Autoradiographs were prepared from sections of 20 oocytes fixed within 5 min of injections aimed at the GV. A common result was that much of the injected DNA was located in an area to one side of the GV (Fig. 1*a*, *b*). In a few cases the labelled DNA was situated exclusively in the cytoplasm. Most significant for this discussion, however, was the fact that in about half of the analysed oocytes, some of the labelled DNA had been deposited clearly inside the GV and had already started to mix with its contents (Fig. 1*c*, *d*).

In the second type of experiment, oocytes whose nuclei contained ^{3}H-SV40 DNA were identified by a method allowing subsequent analysis of the molecular configuration of the injected DNA. Injections of ^{3}H-SV40 DNA were directed either at the GV or into vegetal pole cytoplasm. The GVs were manually removed 24 or 48 h later and ^{3}H-DNA from each GV and cytoplasm was extracted and counted as indicated in the legend to Fig. 2. The results, shown in Fig. 2, demonstrate three points. First, from each oocyte the total radioactivity recovered in acid insoluble form was variable, but the mean for all injected oocytes was almost the same as the total injected radioactivity. Similar recoveries were obtained from oocytes after injection both into the nuclear region (Fig. 2*a*) and into vegetal pole cytoplasm (not shown). Second, ^{3}H-DNA injected into the cytoplasm did not penetrate the GV, even after 48 h (Fig. 2*c*). Third, oocytes injected so as to aim for the GV fell into two classes (Fig. 2*b*). In about half, more than 95% of the recovered DNA was present exclusively in the cytoplasm, a distribution similar to that in oocytes intentionally injected into the cytoplasm. Clearly in these oocytes the injection had either missed the GV or completely leaked out of it, and we refer to them as 'GV-miss' oocytes. In the remainder, referred to as 'GV-hit' oocytes, substantial quantities of ^{3}H-SV40 DNA were present in the GV, in most cases exceeding 80% of the total recovered DNA.

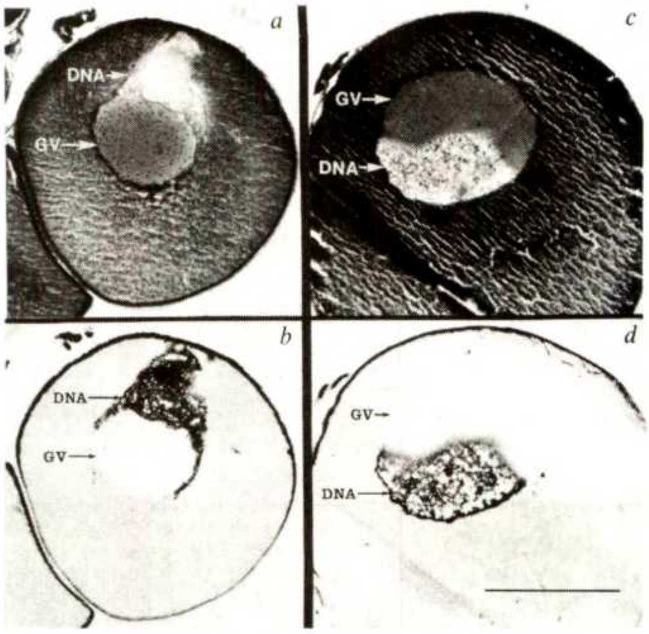

Fig. 1 Intracellular distribution of ^{3}H-SV40 DNA immediately after injection into oocytes. ^{3}H-SV40 DNA (50 nl containing 5 ng) was injected into oocytes[12], aiming for the GV. They were fixed 2–5 min later, for sectioning and staining (*a*, *c*) and subsequent autoradiography (*b*, *d*). In the oocyte shown in *a* and *b*, the injected DNA is located outside the GV. In the oocyte shown in *c* and *d*, the injected DNA is clearly located within the GV, and is already beginning to mix with its contents. Scale bar, 400 μm.

Finally we investigated the configuration of injected DNA recovered from isolated GVs and cytoplasms by methods described in the legend to Fig. 3. Each GV-injected oocyte was classified as 'GV-hit' or 'GV-miss' by counting aliquots of the extracts of nucleus and cytoplasm as described above, and the configuration of the DNA in each oocyte was then correlated with its intracellular distribution. At the time of injection most of the ^{3}H-SV40 DNA was covalently closed and supercoiled (form I), with a small proportion in nicked circular form (form II) (Fig. 3*a*).

DNA deposited directly in the cytoplasm of oocytes was always degraded. It appeared in gels as faint but discrete bands corresponding to the full length linear (form III) and nicked circular (form II) configuration, together with smears representing partially digested linear molecules (Fig. 3*e*); form I DNA was never observed. The absence of any residual covalently closed DNA was confirmed by alkali sucrose gradient analysis of pooled aliquots from these cytoplasms. In most 'GV-miss' oocytes, the DNA recovered from cytoplasm showed a gel distribution closely similar to that of oocytes intentionally injected into the cytoplasm: it was extensively degraded (Fig. 3*d*).

'GV-hit' oocytes showed a strikingly different pattern. First, all the intranuclear ^{3}H-SV40 DNA appeared in gels as a single, sharp form I band; no linear DNA was ever observed (Fig. 3*b*). Second, detectable quantities of ^{3}H-SV40 DNA were sometimes found in the cytoplasms of these oocytes and it is of special interest that in such cytoplasms almost all the ^{3}H-DNA was also in a supercoiled (form I) configuration (Fig. 3*c*). Nicked and linear forms of ^{3}H-DNA were absent or present only in traces. Alkali sucrose gradient analysis of pooled aliquots of 'GV-hit' cytoplasms confirmed the presence of conserved covalently closed ^{3}H-SV40 DNA.

These results show that DNA deposited inside the GV was conserved in supercoiled form, whereas DNA injected directly into cytoplasm was degraded. We interpret the unexpected conservation of DNA in the cytoplasm of 'GV-hit' oocytes as due to leakage of nuclear contents into the cytoplasm. There is direct evidence that such leakage occurred: visibly ruptured GVs were recovered from six GV-injected oocytes, all of which showed form I DNA in their cytoplasm. It seems probable that GV rupture was caused by the large volume of DNA solution injected (50 nl, that is, approximately 1.4 times the volume of the full grown oocyte GV).

Two conclusions may be drawn from these experiments. First, conservation of injected DNA is due, not to its separation from cytoplasmic nucleases by some impermeable membrane, but to a component which renders it stable even in the presence of nuclease. This is demonstrated by the conservation of DNA within the cytoplasm of oocytes in which the GV had been ruptured by injection (cytoplasm analysis of 'GV-hit' oocytes): this conclusion concords with the observed presence of endonucleases within eukaryotic nuclei[3]. Second, the oocyte component responsible for DNA stability is normally localised in the GV.

At this stage, we can make only general observations about the nature of this GV component. It could be a nuclease inhibitor, or a type of molecule which combines with DNA so as to render it resistant to nucleases. We have found that, when conserved, the injected DNA is transcribed[4,5] and is assembled into a nucleoprotein complex whose buoyant density and regularly repeated substructure are those expected of chromatin (A.H.W., J.B.G. and R. A. Laskey, in preparation). However, assembly into chromatin might be a consequence, rather than cause, of DNA stability. In addition to histones[6,7] several other proteins are strongly localised in the GV of oocytes[8,9], and might stabilise DNA.

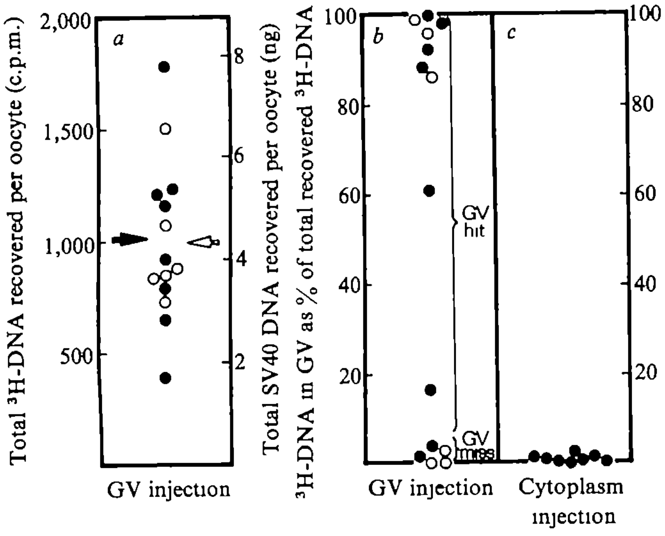

Fig. 2 Location of ^{3}H-SV40 DNA recovered from single oocytes 24 h (○) or 48 h (●) after injection. (*a*) shows, for each oocyte, the total recovered ^{3}H-DNA counts (and hence the total mass of recovered SV40 DNA), taking GV and cytoplasm together. The left hand arrow indicates the mass and radioactivity of DNA in each injection, estimated by delivering the usual oocyte injection volume through the microinjection pipette directly on to a glass chip which was then counted in scintillant. The right-hand arrow shows the mean recovered mass of DNA from these oocytes. (*b*) Refers to oocytes injected aiming for the GV, and (*c*) to oocytes injected in vegetal pole cytoplasm; in both *b* and *c* the counts in each GV are expressed as a percentage of the total counts recovered from the oocyte. Oocytes were injected with approximately 50 nl medium containing 5 ng purified ^{3}H-SV40 DNA, specific activity 380,000 d.p.m. μg^{-1}. After incubation at 19 °C in MBS-H medium[12] for 24 or 48 h, follicle cells were manually peeled off and the GVs were manually removed and washed free of yolk. Each GV and cytoplasm was frozen within 30 s of isolation. ^{3}H-DNA was extracted by making a homogenate of each GV or cytoplasm in 100 μl buffer containing 50 mM Tris, *p*H 7.5, 5 mM EDTA, 0.5% sodium dodecyl sulphate, 0.75 mg ml^{-1} proteinase K and 0.8 mg ml^{-1} yeast tRNA (as carrier). The homogenate was incubated at 37 °C for 2 h and extracted twice in chloroform : isoamylalcohol (24 : 1). The aqueous phase was precipitated in 10% trichloroacetic acid at 0 °C on to Millipore filters which were dried and counted with an efficiency of about 60% in scintillant.

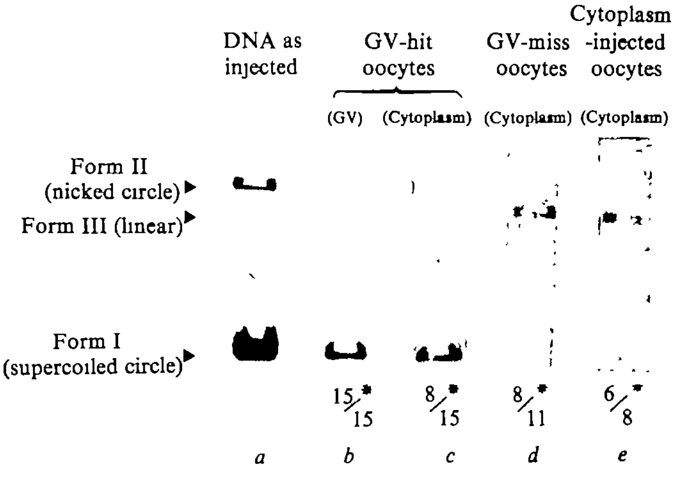

Fig. 3 Conformation of DNA injected into oocytes and recovered from isolated GVs and cytoplasms. GV-injected oocytes, classed as 'GV-hit' or 'GV-miss' as described in Fig. 2, are compared with oocytes injected in vegetal pole cytoplasm. The gel tracks show typical examples of the conformation adopted by ^{3}H-SV40 DNA in the GV of 'GV-hit' oocytes and in the cytoplasm of oocytes of all classes. GVs of 'GV-miss' and cytoplasm-injected oocytes contained neglible amounts of ^{3}H DNA and were not analysed on gels. Seven of fifteen cytoplasms from 'GV-hit' oocytes and two of eight cytoplasms from cytoplasm-injected oocytes differed from the pattern shown in that they contained little ^{3}H DNA and showed no discrete gel bands. Three of eleven cytoplasms of 'GV-miss' oocytes also differed from the pattern shown in that they contained form I DNA. At the time of isolation the GV of one of these oocytes was found to be merely a ruptured membranous sac and we presume that, in the other two cases also, GV material had leaked into cytoplasm. Individual GVs or cytoplasms were homogenised and extracted in chloroform: isoamylalcohol as described in the legend to Fig. 2. An aliquot was removed for scintillation counting to permit GV-injected oocytes to be classified as 'GV-hit' or 'GV-miss', and the remainder was loaded on 1% Agarose slab gels for electrophoresis[13] at 40 mA for 14 h. Thereafter the gel was impregnated with PPO, dried and fluorographed[14]. *Proportion of samples with the gel pattern shown

The behaviour of this GV component at nuclear division is of interest. It is probably released into the cytoplasm when the oocyte nuclear membrane breaks down at the time of maturation into eggs; uncleaved eggs, unlike oocytes, conserve DNA injected into their cytoplasm[10]. This type of component may, we suggest, be of general occurrence in eukaryotic cells, protecting chromosomal DNA from nuclease attack throughout the cell cycle and especially during mitosis.

Conceivably, cytoplasmic nucleases could be of value to cells, ensuring the breakdown of any exogenous DNA taken into the cell. Uptake of exogenous DNA occurs rather frequently as a result of cell death. in the course of the normal cell renewal in many tissues, the products of dead cells—including their DNA—are ingested by their viable neighbours[11]. Phagocytes also may require protection from ingested bacterial DNA. Viral DNA however may be resistant to degradation by virtue of its own core proteins.

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Rigid control of synthesis of collagen Types I and III by cells in culture

THE constituents of the extracellular matrix have a fundamental role in the structure and function of all tissues. A major insight into the composition of this matrix came with the discovery[1-3] that interstitial collagen was not homogeneous, but rather composed of two species, termed Type I and Type III. Type I collagen (composed of two $\alpha 1(\mathrm{I})$ chains and one $\alpha 2$ chain) usually forms densely packed fibrils (the cross-banded collagen commonly seen in electron micrographs). In comparison, Type III collagen (composed of three $\alpha 1(\mathrm{III})$ chains) exists, in part, as reticulin, in which fine, randomly dispersed collagen fibrils are intimately associated with other connective tissue components[3,4]. Appreciation of the possible functional significance of the heterogeneity of the interstitial collagens has come from the analysis of the ratio of Type I to Type III collagen in different tissues in health and disease. Most pliable tissues (skin, intestine, blood vessels, lung) have a I : III ratio of 2–3 to 1, while rigid tissues (bone, tendon) contain only Type I collagen[5,6]. Studies of inflammation and wound healing have demonstrated that early granulation tissue (a material with little tensile strength) has a very low I : III ratio[4,7], while mature scar (a stiff tissue) has a high I : III ratio[8]. In addition, disorders associated with a loss of tissue compliance (arterosclerosis[9], pulmonary fibrosis[10]) are associated with a shift in the normal I: III ratio toward relatively more Type I collagen. Taken together, these findings suggest that the structure and function of tissues are intimately associated with the relative amounts of the interstitial collagens, with Type I tightly packed and tough and Type III loosely associated and pliable. If the relative amounts of collagens I and III are as important as this data suggests, the maintenance of normal interstitial tissues must be critically dependent on cells in the local environment rigidily maintaining their differentiated state with respect to the synthesis and degradation of these collagen types. We describe here a study designed to evaluate this hypothesis, using a model system to assess the 'tightness' of cellular control of the synthesis of collagens I and III by a population of similar cells (fibroblasts in culture) over approximately 20 replicative cycles.

To accurately assess the relative rates of synthesis of Types I and III by cells in culture, we adapted cyanogen bromide (CNBr) mapping techniques to quantitate all of the collagen in the culture, regardless of its type, form or location. To increase the accuracy of the quantitation of both collagen types, we used NB-6, a diploid fibroblast derived from the newborn rabbit lung, since it synthesises

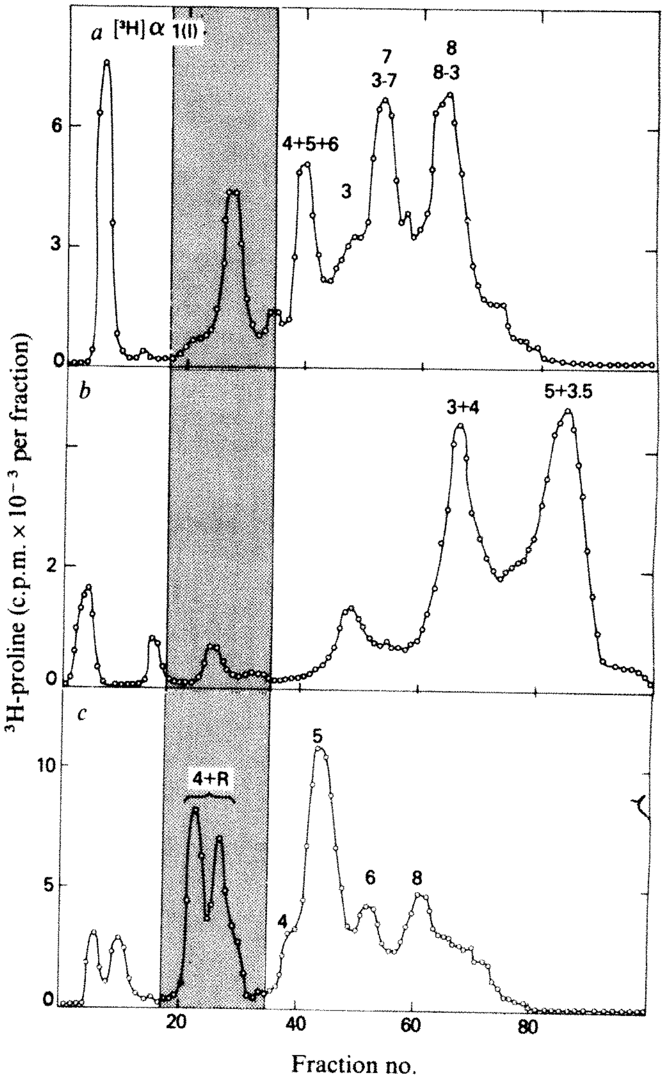

Fig. 1 Quantitation of the ratio of Type I to Type III collagen synthesised by rabbit lung fibroblasts (NB-6) in culture, step 1: carboxymethyl-cellulose (CMC) chromatography of CNBr peptides. NB-6 cells were grown to confluency in 100 mm² plates, rinsed with phosphate-buffered saline (PBS), and labelled for 24 h at 37 °C in Dulbecco's modified Eagle's medium (DMEM) 10 ml per plate, containing 0.3 mM ascorbic aicd, 0.5 mM β-aminoproprionitrile and either 50 μCi ^{14}C-proline (260 mCi mmol^{-1}) per plate or 1.5 mCi ^{3}H-proline (32 Ci mmol^{-1}) per plate. Isolation, morphological characterisation and subcultivation of the NB-6 culture was as previously described[11]. Medium was collected, dialysed against 10 mM Tris-HCl, *p*H 7.4, 2 M urea, and ^{3}H-proline labelled Type I and Type III collagens were prepared using diethylaminoethylcellulose chromatography[11,16]. The ^{3}H-proline labelled Type I collagen was further purified into ^{3}H-α1(I) and ^{3}H-α2 chains by CMC chromatography[17]. Using these methods, the labelled Type III collagen, Type I collagen and α1(I) and α2 chains are greater than 98% pure[11]. To approximate the conditions in which the collagens in an entire tissue culture plate would be quantitated, labelled collagens were added to pooled cells and medium from five unlabelled culture plates, dialysed against 0.1 M acetic acid, lyophilised and CNBr peptides were produced using an excess (100 mg) of CNBr. The CNBr peptides of the purified collagens or their component chains were separated by CMC chromatography (0.9 × 12 cm column; 250 ml × 250 ml, 10 mM to 160 mm NaCl gradient in 20 mM Na-citrate, *p*H 3.8, flow rate 40 ml h^{-1}; 5 ml fractions) and aliquots (0.2 ml) of each fraction were counted in Aquasol. *a*, CNBr peptides of ^{3}H–α1(I) chains synthesised by NB-6 cells in culture; the characteristic peptides are labelled. *b*, CNBr peptides of ^{3}H-α2 chains from the same cultures; α2CNBr (3.5) is probably peptide 3–5; it has a molecular weight[17,18] of approximately 60,000. *c*, CNBr peptides of ^{3}H-Type III collagen. The shaded area ('region A') was used to quantitate the ratio of Type I to Type III collagen synthesised by NB-6.

significant quantities of both collagens I and III (ref. 11).

NB-6 cultures were labelled with ^{3}H-proline in the presence of ascorbic acid and β-aminopropionitrile at subcultivations 5 and 10 at different stages in the cell growth curve. The cultures were labelled for 18 h (in the conditions used, NB-6 cells synthesise collagen in a linear fashion for >24 h) and the resulting ^{3}H-proline labelled proteins solubilised with CNBr. To quantitate the ratio of ^{3}H-Type I to ^{3}H-Type III collagen synthesised by the cultured cells, peptides characteristic of ^{3}H-Type I and ^{3}H-Type III collagens were then isolated by carboxymethyl-cellulose chromatography followed by isoelectric focusing gels (Figs 1 and 2). Region A (shaded area Fig. 1) was chosen for the I: III quantitation for two reasons: (1) it is the least complex region of the Type I and Type III CNBr peptide map, containing only one peptide from Type I [α1(I)CB3] and two peptides from Type III [α1(III)CB4+α1(III)CB(R)] (Fig. 2*a*); and (2) the I and III peptides in region A are clearly separated from each other by isoelectric focusing (Fig. 2*b*); this procedure is quantitative and easily reproducible. The final ratio of ^{3}H-Type I to ^{3}H-Type III synthesised by the cells in culture was determined from a standard curve prepared from known mixtures of ^{3}H-Type I and ^{3}H-Type III previously purified from NB-6 cultures (Fig. 2*c*).

This method obviates a number of technical problems inherent in the quantitation of collagen types synthesised by cells in culture: (1) the use of a standard curve circumvents possible differential losses of I and III during analysis and takes into account the differences in proline+hydroxyproline residues in the I and III peptides used for quantitation; (2) CNBr cleavage of the entire plate ensures that all of the newly synthesised I and III will be quantitated, independent of whether the collagens are in solution in the culture medium, precipitated on the cell layer, or intracellular and also independent of the extent of processing from procollagen to collagen; (3) to ensure that ^{3}H-Type I and ^{3}H-Type III are hydroxylated to the same extent and not contaminated by non-collagen proteins, the isoelectric focusing gels are evaluated by quantitating both ^{3}H-proline and ^{3}H-hydroxyproline in the I and III peptides; and (4) since cross-linked peptides may map differently, β-aminopropionitrile is added to the cultures in concentrations which inhibit cross linking of the newly synthesised collagens[11].

The NB-6 cells synthesise collagen Types I and III in a ratio of 1.94±0.09 (mean ± s.e.m.) to 1 (that is, 66% I, 34% III) over at least 20 replicative cycles (Fig. 3). Thus in conditions in which the extracellular milieu is defined and constant, the fibroblast exhibits remarkably tight control over the relative synthesis of these two collagen types. This holds true even through periods of rapid cell

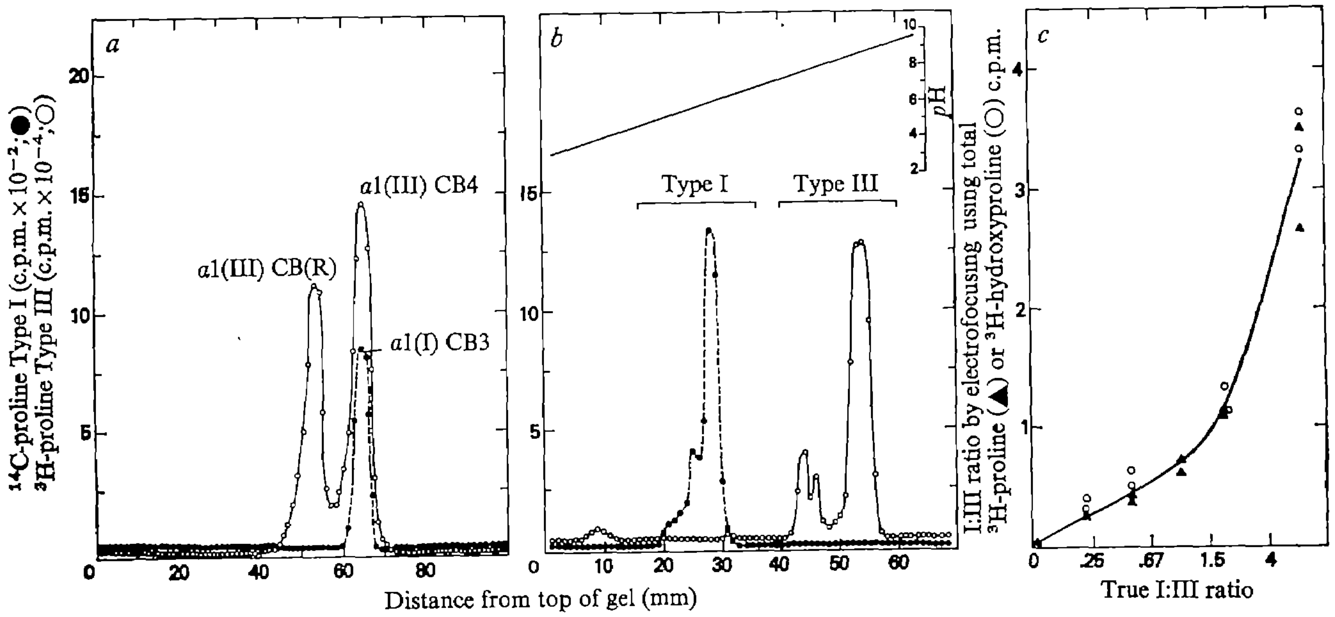

Fig. 2 Quantitation of the ratio of Type I to Type III collagen synthesised by NB-6 cells in culture, step 2: analysis of ^{3}H-labelled CNBr peptides from region A of the CMC chromatogram. A mixture of ^{14}C-Type I and ^{3}H-Type III CNBr peptides were chromatographed on CMC. The A region peptides were precipitated with ethanol (−20°, 18 h, using 500 μg of rabbit skin Type I collagen CNBr peptides as carrier), isolated by centrifugation (72,000*g*, 2 h, 4 °C). *a*, ^{14}C-Type I (●) and ^{3}H-Type III (○) peptides from region A isolated on sodium dodecyl sulphate (SDS)–acrylamide gels. The peptides were dissolved in 1% SDS, 1% β-mercaptoethanol in 0.1 M phosphate buffer, *p*H 7.4 and electrophoresed as previously described[11]. Region A contains one Type I peptide α1(I)CB(3) of approximately 12,000–13,000 molecular weight and two Type III peptides [α1(III)CB4 and α1(III)CB(R)]; α1(III)CB4 electrophoreses with α1(I)CB3 and α1(III)CB(R) electrophoreses with an apparently larger molecularweight. Compared with the α1(I) and α1(III) peptides, there are no significant α2 peptides found in region A. *b*, ^{14}C-Type I (●) and ^{3}H-Type III (○) peptides from region A separated by isoelectric focusing gels. Five-tenths ml of a concentrated acrylamide solution [made up of 3 ml of 30% acrylamide–1% *bis*-acrylamide; 0.3 ml *p*H 3–10 Ampholytes (LKB); 0.7 ml ammonium persulphate (15 mg ml^{-1})]; was added to 1 ml of the A region CNBr peptides at 4 °C, the solution vortexed and used to form a 67×5-mm cylindrical gel. The gels were focused at 1 mA per gel (0.2 M phosphoric acid anode solution, 0.2 M NaOH cathode solution) until 200 V was reached, then 200 V for 5 h total[20]. The *p*H of the focused gels was measured using a micro *p*H electrode (Gel Pro-pHiler, Bio Rad) and confirmed by determining the focusing point of standard proteins[21]. After focusing, the gels were fractionated at 1 mm intervals (Gilson Gel fractionator) and fractions were counted in Aquasol. Alternatively, gels were sectioned at appropriate intervals, hydrolysed in 6 M HCl and the labelled hydroxyproline quantitated[11]. The ^{14}C-Type I peptide [α1(I)CB3] focused at between *p*H 4.5 and 6.5; and ^{3}H-Type III peptides [α1(III)CB4+α1(III)CB(R)] focused between *p*H 7.1 and 8.3. *c*, Various mixtures of ^{3}H-Type I collagen and ^{3}H-Type III collagen synthesised by NB-6 cultures were treated with CNBr along with a fixed amount of ^{14}C-Type III collagen (used to locate region A on CMC) and unlabelled cells and medium from five culture plates. Each mixture was chromatographed on CMC, region A pooled (as determined from the elution pattern of ^{14}C-Type III; see Fig. 1*c*) ethanol precipitated and electrofocused. The Type I region (*p*H 4.5–6.5) and Type III region (*p*H 7.1–8.3) was fractionated and the ^{3}H-proline and ^{3}H-hydroxyproline d.p.m. quantitated. Abcissa: the known ratio of ^{3}H-Type I and ^{3}H-Type III mixed together before CNBr cleavage. Ordinate: the I:III ratio quantitated with isoelectric focusing of CMC region A: ▲, total ^{3}H c.p.m. in the Type I region/total ^{3}H c.p.m. in the Type III region; ○, ^{3}H-hydroxyproline in the Type I region/^{3}H-hydroxyproline in the Type III region. Similar results are obtained using either total ^{3}H c.p.m. or ^{3}H-hydroxyproline. In an actual experiment quantitating the ratio I:III synthesised by NB-6 in culture, the c.p.m. of ^{3}H-hydroxyproline in the Type I region is compared to the c.p.m. of ^{3}H-hydroxyproline in the Type III region and the true I:III ratio read off the abcissa of the standard curve.

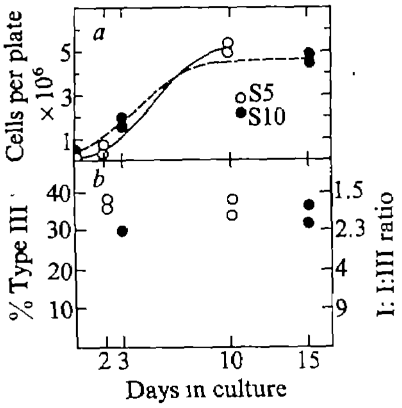

Fig. 3 Quantitation of the ratio of Type I to Type III collagen synthesised by NB-6 cells at two different subcultivations (S5 and S10) and at different times within each subcultivation (early log through late confluency). NB-6 cells in the 4th and 9th subcultivation were detached, counted and plated at a density of 2×10^5 cells per 100-mm² plate in 25 ml DMEM containing 10% foetal calf serum, 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and 5 μg ml^{-1} fungizone. The medium was replaced every 4th day. For quantitation of the ratio of Type I to Type III collagen synthesised, the NB-6 cells were incubated for 18 h at 37 °C in 4 ml DMEM containing 0.3 mM ascorbic acid, 0.5 mM β-aminopropionitrile and 500 μCi ^{3}H-proline (32 Ci mmol^{-1}). After incubation, the cells were detached by scraping the plates, the cells and medium from five plates were combined, dialysed against 0.1 M acetic acid and lyophilised. Thereafter, the methods described in the legends to Figs 1 and 2 were used. An average of 90% of the ^{3}H-hydroxyproline in the plates was solubilised with CNBr. As a check on the number of cells replicating in the plates at each time, separate plates were incubated under identical conditions with 10 μCi per plate of ^{3}H-methylthymidine (65 Ci mmol^{-1}) for 1 h at 37 °C. After incubation, the cells were washed with PBS, detached, counted and the incorporated ^{3}H-thymidine quantitated. For subcultivation 5, cells at day 10 incorporated 0.1% ^{3}H-thymidine per cell of that incorporated by cells at day 2; for subcultivation 10, cells at day 15 incorporated 0.4% ^{3}H-thymidine per cell as did cells at day 3. *a*, Cell number at early log (days 2–3), late log-early confluency (day 10) and late confluency (day 15) are shown for cells in subcultivation 5(○) and 10 (●). *b*, The ratio of Type I-to-Type III collagen synthesised by NB-6 cells at the same subcultivations and days in each subcultivation as in (*a*). Within the limits of the method, the ratio of I:III was invariant at the times studied with an average of % Type III of 34.1±1.0 (I:III 1.94±0.09).

division. For example, on day 2 of passage 5 the incorporation of ^{3}H-thymidine per cell was 1,000-fold greater than at day 10 of passage 5; even so, the I : III ratio of newly synthesised collagens was identical at both days (Fig. 3). Since 20 population doublings should be ample time for different clonal subpopulations to become apparent[12], these results also suggest, at least for NB-6, all cells in the culture are synthesising collagens I and III at approximately the same ratio. If different clones are characterised by different I : III ratios, the relative number of these clones remains constant in conditions of a defined extracellular milieu.

These results do not imply that fibroblasts never alter the relative amounts of collagen types that they synthesise. In special circumstances when cells become senescent[13,14], or when the extracellular milieu changes, such as in inflammation[15], these cells could change the relative proportions of collagens I and III that they produce, either by altering the differentiated characteristics of each cell or by selecting specific clones emphasising one collagen type. The data presented, however, indicate that in conditions of a constant extracellular environment, the relative ratio of the two known interstitial collagens synthesised by a population of fibroblasts is invariant. Thus, the cells most concerned with the maintenance of interstitial structures are programmed to preserve the status quo by continuing to synthesise the major interstitial components in a carefully controlled fashion.

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Long term culture of tumour-specific cytotoxic T cells

MANY investigators have been successful in the maintenance of long term tissue culture of human bone marrow-derived (B) cells. These cell lines have been established from both normal subjects[1] and from patients with lymphoproliferative disorders[2]. In most cases, long term B-cell lines have been shown to harbour the Epstein–Barr virus genome which some investigators feel is required for establishment and maintenance of long-term cultures[3]. There are fewer reports describing continuous culture of human thymus derived (T) cell lines, and when successful, the lines have only been established from patients with acute lymphocytic leukaemia[4]. Although these cell lines have been shown to bear surface markers of normal human T lymphocytes, there have been no reports which suggest that they possess the ability to respond to immunologic stimuli or to differentiate into antigen-specific lymphocytes. Cytotoxic murine T cells have been kept in continuous culture only through repetitive mixed-lymphocyte stimulation[5]. In contrast to long term human lymphocyte lines, these cells proliferated only when stimulated with allogeneic lymphocytes and eventually died after a few weeks in culture. Morgan, Ruscetti and Gallo recently reported a method by which medium conditioned by phytohaemagglutinin-stimulated normal human lymphocytes allowed for the selective long-term growth of normal T cells[6]. In contrast to the previously mentioned cell lines, the proliferation of these reported T-cell cultures was totally dependent on the presence of an exogenously-produced growth factor supplied by the conditioned medium. In this report we describe an adaptation of this method which allows the long term culture of antigen-selected cytotoxic T cells which continue to demonstrate high levels of syngeneic tumour-specific cytotoxicity after more than 4 months in culture.

Cytotoxic T cells were generated in secondary allogeneic mixed tumour–lymphocyte cultures (MTLC). Spleen cells were harvested from normal C57Bl/6 mice and from C57Bl/6 mice immunised with allogeneic (*H-2*^{d}) F4-5 Friend virus (FLV)-induced leukaemia cells (from Dr W. Ostertag, Max Planck Institute, Gottingen, Germany). Both spleen cell populations were co-cultured with mitomycin-treated (100 μg ml^{-1} for 60 min)[7] F4-5 cells in Click's medium (Altick Associates) with 2% heat inactivated foetal calf serum (FCS) for 5 d (responder: stimulator ratio of 40 : 1). Viable cells collected from primary allogeneic MTLC were restimulated with mitomycin-treated F4-5 cells and placed back in culture for a further 2 d. Cultures of cytotoxic cells generated in secondary allogeneic MTLC of normal C57B1/6 spleen cells were designated cytotoxic lymphoid line 1 [(CTLL(1)] and cells collected from repetitive allogeneic MTLC of spleen cells from C57B1/6 mice immunised with F4-5 were designated CTLL(2). The population of cytotoxic lymphocytes collected from secondary allogeneic MTLC was a heterogeneous one in that one lymphoid subpopulation was reactive against allogeneic targets while a second subpopulation contained lymphocytes capable of effecting syngeneic tumour-specific cytotoxicity[13].

Viable cells collected from secondary allogeneic MTLC were placed in continuous culture in 50% Click's medium and 50% growth factor. Growth factor was prepared from 48-h tissue culture medium (RPMI 1640 (GIBCO) with 10% FCS) from cultures of concanavalin A (con A, 2.5 μg ml^{-1}, Miles–Yeda) stimulated normal DBA/2 spleen cells. Growth factor was filtered through a 0.2-μm filter (Nalge–Sybron) to remove subcellular particles before use in long-term culture medium.

Both CTLL(1) and CTLL(2) have remained in continuous proliferative culture for 22 weeks. Representative growth curves for both lines are shown in Fig. 1. Cells seeded at a concentration of 5×10^3 per ml reach a saturation density of approximately 3×10^5 cells per ml after 6 or 7 d in culture thereby demonstrating a doubling time of 24 h. Proliferative growth is totally dependent on the presence of growth factor. CTLL(1) and CTLL(2) cannot be maintained in continuous culture in either Click's medium supplemented with FCS or in RPMI 1640 medium supplemented with FCS and con A.

Cells from cultures of CTLL(1) and CTLL(2) were used periodically as effector cells in lymphocyte-mediated cytolysis (LMC) assays[8], against the allogeneic tumour target cell, F4-5 (Fig. 2) and the syngeneic FLV-induced FBL-3 (Hn), (Fig. 3). Both F4-5 and FBL-3 (Hn) were confirmed as non-virus-producing murine leukaemia cells by reverse transcriptase[9] and X-C assays[10]. When LMC data is expressed in terms of lytic units per 10^6 effector cells (LU $\times 10^{-6}$), the allogeneic cytotoxicity mediated by CTLL(1) increased from 76.9 LU $\times 10^{-6}$ after 3 weeks in culture to 555 LU $\times 10^{-6}$ after 17 weeks in culture. Similarly, allogeneic lysis effected by CTLL(2) rose from 40 LU $\times 10^{-6}$ after 3 weeks of culture to 500 LU $\times 10^{-6}$ 14 weeks later. Results of LMC assays (Fig. 3) indicate that the syngeneic tumour cell lysis mediated by CTLL(1) and CTLL(2) was tumour specific. Although FBL-3 (Hn) murine leukaemia cells were effectively lysed, syngeneic normal cells (C57B1/6 lymph node and con A-stimulated (2.5 μg ml^{-1}) spleen cells remained unaffected). Syngeneic tumour-specific cytotoxicity demonstrated by CTLL(1) effector cells increased from 10 LU $\times 10^{-6}$ after 3 weeks in culture to 333 LU $\times 10^{-6}$ after 17 weeks in culture. CTLL(2)-mediated cytotoxicity of FBL-3 (Hn) cells rose from 16.7 LU $\times 10^{-6}$ following 3 weeks in culture to 142.9 LU $\times 10^{-6}$ after 17 weeks of continuous *in vitro* passage.

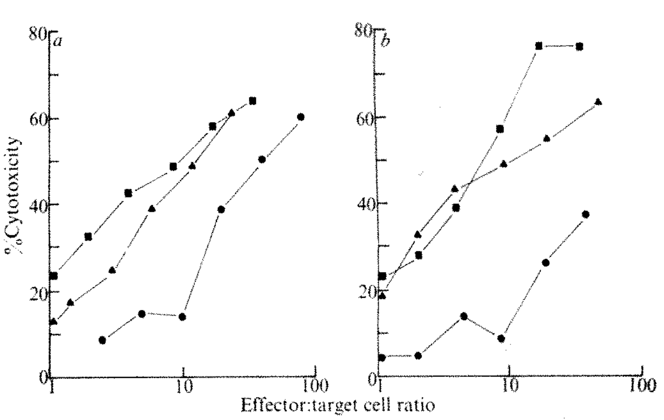


Fig. 2 *a*, Allogeneic cytotoxicity demonstrated by CTLL(1) against the FLV-induced non-producer murine leukaemia cell, F4-5, after 3 weeks (●), after 10 weeks (▲), and after 17 weeks (■), in continuous culture. *b*, Cytotoxicity demonstrated by CTLL(2) against F4-5 after 3 weeks (●), 10 (▲) and 17 weeks (■) in culture. Per cent cytotoxicity was determined in lymphocyte-mediated cytolysis assays which measured Cr^{51} release of radiolabelled target cells. Target cells were labelled with 250 μCi of Na$_2$Cr^{51}O$_4$ (specific activity 100–400 mCi per mg Cr, Amersham–Searle). Target cell concentrations were adjusted in RPMI 1640 with 10% FCS to 0.1 c.p.m. per cell. Target cells (100 μl) were mixed with log$_2$ dilutions of effector cells collected from CTLL(1) and CTLL(2) long term cultures, in V-bottom microplates (1S MVC-96-TC, Linbro Scientific). The Cr-release reaction was stopped after a 4-h incubation at 37 °C by a 10-min 300*g* centrifugation at 4 °C. c.p.m. of Cr-release were determined by counting 100 μl of each well supernatant on a liquid scintillation counter, per cent specific lysis was determined using the following equation

$$\%\text{Specific lysis} = \frac{\text{experimental c.p.m.} - \text{medium control c.p.m.}}{\text{maximum release c.p.m.} - \text{medium control c.p.m.}} \times 100$$

Medium control c.p.m. were obtained from 100-μl supernatant aliquots sampled from microplate wells containing 100 μl of target cells and 100 μl of RPMI 1640 with 10% FCS. Maximum release c.p.m. were generated from microplate wells containing 100 μl of target cells and 100 μl of Zap Isoton Solution (three drops added to 5 ml double distilled H$_2$O), Coulter Electronics, Lytic units were defined as the number of effector cells necessary to cause 30% specific lysis. Growth factor was not present in LMC assays.

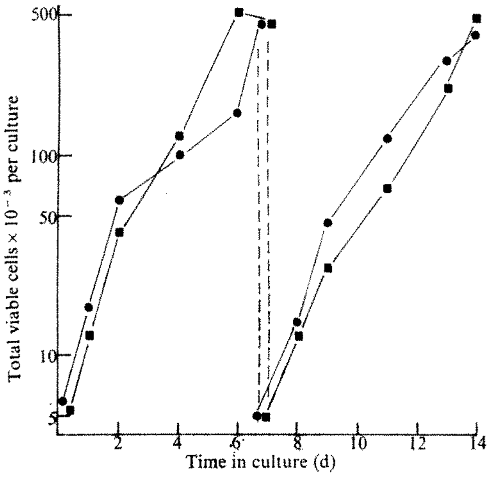


Fig. 1 Representative growth curves of CTLL(1) (●) and CTLL(2) (■). Dotted lines indicate passage of long term cell lines and reseeding of additional culture flasks at the day 0 cell concentration of 5×10^3 cells per ml. Both cell lines exhibited this pattern of proliferative growth provided they were grown in the presence of medium containing 50% growth factor. Click's medium with 50% growth factor was also supplemented with 2% heat-inactivated FCS, 300 μg ml^{-1} fresh L-glutamine, 50 units ml^{-1} penicillin, 50 μg ml^{-1} streptomycin, 16 μmol ml^{-1} NaHCO$_3$ and 25 μmol ml^{-1} HEPES buffer. Cells were cultured in a humified atmosphere of 5% CO$_2$ in air at 37 °C.

Figure 4 shows Wright–Giemsa-stained preparations of CTLL(1) and CTLL(2) after 22 weeks of continuous proliferation *in vitro*. Both cell lines are blastoid and highly vacuolar in appearance, and were lysed by anti-theta serum (AKR anti-C3H thymus from Litton Bionetic) when tested using absorbed rabbit complement[11]. Histochemical stains of both CTLL(1) and CTLL(2) for peroxidase, periodic acid Schiff, napthal chloroacetate and α-napthylacetate esterases, were negative. These tests confirm that the syngeneic tumour-specific cytotoxicity demonstrated by CTLL(1) and CTLL(2) was mediated by cytotoxic T cells maintained *in vitro* with the aid of a growth factor produced by con A-stimulated spleen cells.

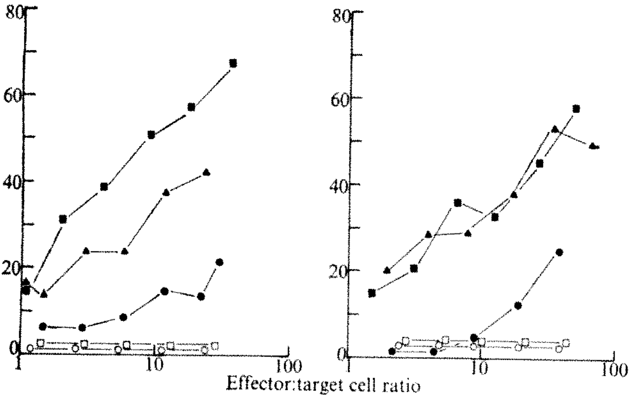
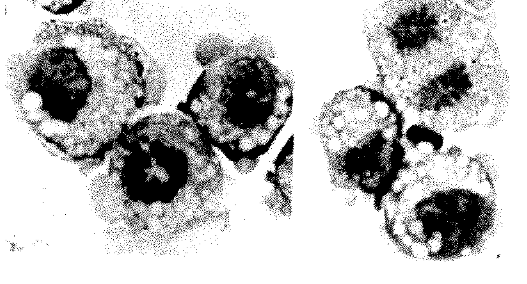


Fig. 3 *a*, Cytotoxicity of syngeneic FBL-3 (Hn) Friend leukaemia cells effected by CTLL(1) cells after 3 (●), 10 (▲) and 17 weeks (■) in culture. Cytotoxicity demonstrated by CTLL(1) against syngeneic normal lymph node (○) and con A-stimulated spleen cells (□). *b*, Cytotoxicity of FBL-3 (Hn) by CTLL(2) cells after 3 (●), 10 (▲), and 17 weeks (■) in culture. CTLL(2)-mediated cytolysis of syngeneic normal lymph node (○) and con A-stimulated spleen cells (□). FBL-3 (Hn) was adapted to continuous *in vitro* culture from ascites-passaged FBL-3 cells, obtained from Dr Ronald Herberman, NCI. LMC assay conditions, and radiolabelling of all target cells identical to those described in Fig. 2.

The continuous proliferation of differentiated T lymphocytes *in vitro* raises questions as to the regulatory factors operating *in vivo* which prevent continuous clonal expansion after antigenic stimulation. The demonstration that continuous proliferation of cytotoxic T cells *in vitro* is possible, suggests that some humoral or cellular factor may be operating *in vivo* to prevent differentiated proliferation. Furthermore, the long term culture system described here might serve as a starting point for the identification and characterisation of these growth promoting and inhibiting, regulatory factors.

Finally, although cytotoxic lymphocytes generated *in vitro* have beeen shown to be effective mediators of tumour cell lysis *in vivo*[12], the inability to generate these cells in large numbers has prevented their widespread use. The ability to propagate large populations of syngeneic tumor antigen-specific cytotoxic lymphocytes through the use of a growth factor, makes possible the testing of these lymphocytes in adoptive immunotherapy. The syngeneic cytotoxic specificities of the long-term T-cell lines tested in this study were directed against non-producer leukaemia cells. Because murine non-producer leukaemia cells represent the model system most analagous to human leukaemia, this study provides evidence that the *in vitro* generation of large numbers of leukaemia-specific human cytotoxic T cells might be possible.

Fig. 4. Wright–Giemsa-stained cytocentrifuge preparations of *a*, CTLL(1) and *b*, CTLL(2) cells after 22 weeks of continuous culture *in vitro*. (×6,000).



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Effect of glucose and insulin on collagen secretion by human skin fibroblasts *in vitro*

Increased glucose concentration in the medium in which normal fibroblasts are grown in culture caused an increase in one type of procollagen synthesised, an increase in the incorporation of labelled carbon from glucose into collagen, and an increase in the rate of collagen secretion. The addition of insulin to the culture media did not affect collagen secretion.

Skin fibroblasts growing *in vitro* synthesise collagen when the medium is supplemented with ascorbic acid[1]. Most of this collagenous protein remains soluble in the medium as procollagen types I and III (refs 2–5). The procollagens are secreted by the fibroblasts into the medium where they are eventually cleaved, polymerised and deposited as extracellular insoluble collagen fibrils. Human fibroblasts *in vitro* do not efficiently convert procollagen into collagen, so there is an accumulation of intact procollagen in the medium[6].

Skin biopsy material was obtained from human subjects undergoing surgery for non-metabolic disorders. Fibroblasts were isolated after trypsinisation, and grown as monolayers in minimum essential medium (MEM, Flow Labs) plus 10% foetal calf serum. Ascorbic acid (50 μg ml^{-1}) was added to the culture medium to ensure maximal collagen synthesis[7]. Cells were continuously passaged and monitored periodically for their ability to incorporate tritiated thymidine into DNA (ref. 8). All the experiments reported here were performed on cells at confluency. For 24 h before the experiment, the cells were placed in a serum-free medium (MEM) plus 1% non-essential amino acids. After 24 h, fresh MEM + 1% non-essential amino acids were added and glucose and/or insulin (0.1 U ml^{-1}) was added to the experimental dishes. The medium was removed 24 h later and analysed for collagen-like material according to the technique of Paz and Gallop[9]. Briefly, collagen was isolated by TCA precipitation, hot TCA solubilisation and collagenase (purified by chromatography)[10] treatment and determined by microbiuret analysis using rat-tail collagen as standard. In some experiments, hydroxyproline was determined by the method of Bergman and Loxley[12].

To demonstrate that the material isolated in our assay system contained collagen or procollagen, samples were collected after collagenase digestion, desalted by cation exchange column, and hydrolysed in 6 M HCl for 24 h at 100 °C. Amino acids were analysed on a Beckman 121 M amino acid analyser. In selected experiments, protein labelled with ^{3}H-proline was characterised by DEAE-52 cellulose chromatography[13]. Cells remaining in the dish after removal of the medium were washed, solubilised with alkali, and protein[14] and DNA[15] determinations carried out.

Cells growing in a high glucose medium secreted more collagen than those in glucose at a physiological concentration (Table 1). The addition of insulin did not increase the synthesis or secretion of collagen at glucose concentrations of 1 mg ml^{-1} or 3 mg ml^{-1} in the medium. Insulin did, however, stimulate the accumulation of non-collagen protein in the monolayer cells. We conclude that the increase in cell protein (data not given) is part of an insulin stimulation of growth. In the range of glucose concentrations of 1–5 mg ml^{-1}, increasing amounts of collagen were found in the media of the fibroblasts, but above this range no further increase was noted (Table 2).

DEAE-52 cellulose chromatography of the labelled medium collected after 24 h of high and normal glucose treatment gave a radioactive profile similar to that described by others[2,3,5,16]. Normally we find three peaks; first a very small peak which is native collagen, a second large peak which is procollagen type I and a third peak which is presumably procollagen type III. In glucose-treated cultures there is a substantial (30–50%) increase in the third peak. Salt-extracted ^{3}H-labelled collagen from the cellular material remaining attached to the culture chamber gave identical DEAE-52 profiles with 24 h of 1 mg ml^{-1} or 3 mg ml^{-1} glucose treatment.

Table 1 Effect of glucose and insulin on the amount of collagen secreted by fibroblasts into the medium

Experiment	Control	Glucose (3 mg ml^{-1})	Insulin (0.1 U ml^{-1})	Gluscose + insulin
1	0.066	0.115	0.047	0.100
2	0.131	0.193	0.141	0.197
3	0.152	0.207	0.185	0.227
4	0.102	0.139	0.053	0.147
5	0.086	0.182	0.068	0.167
Mean	0.107	0.167	0.097	0.168
± s.e.m.	0.015	0.017	0.027	0.022

The amount of collagen secreted into the serum-free medium by fibroblasts during a 24-h period expressed as μg collagen per μg monolayer cell protein in each culture dish Collagen was measured in duplicate by microbiuret assay against rat-tail collagen. Total monolayer cell protein per dish was determined by dissolving the monolayer in alkali and using an aliquot of this in standard protein determination against bovine serum albumin. In these experiments the amount of protein in each culture dish remained fairly constant, the amount of collagen increased by 50% after the addition of glucose. Insulin had no effect. The data are the mean of five experiments ± the standard error of the mean.

Amino acid analysis of extracted collagen demonstrated twice as much hydroxyproline in each sample of the high glucose medium than in the normal. Colorimetric analysis for hydroxyproline gave similar results; for example 24 μg collagen in the control medium and 36 μg collagen in the glucose-treated medium.

We wondered whether the increased secretion was a result of increased cell proliferation, however, in our growth conditions neither the incorporation of tritiated thymidine in DNA nor the total DNA content was increased by raising the glucose concentration of the medium. In addition, there was no visible evidence (using phase-contrast microscopy) of increased proliferation of fibroblasts in high glucose media. There was no significant difference in cell protein or cell counts with increased glucose concentration in the medium. Rheinwald and Green[17] also reported no increase in cell density at high glucose levels.

Table 2 Effects of glucose concentration on collagen secretion by fibroblasts

Experiment	Glucose concentration (mg ml^{-1})				
	1	2	3	5	7
1	0.049		0.081	0.131	0.149
2	0.058		0.080		
3	0.072	0.108	0.138		

The effect of glucose concentration on the amount of collagen secreted into the medium by fibroblasts, expressed as μg collagen in the medium per μg monolayer cell protein as described in Table 1. There is a dose-dependent response of human diploid fibroblasts to increased glucose concentration in the medium. At glucose concentration of 5 mg ml^{-} or higher, the stimulation of collagen accumulation in the medium began to level off. The amount of protein associated with the monolayer remained constant.

In one experiment, the incorporation of ^{14}C from glucose into collagen was measured. The amount of label incorporated into collagen was 85% greater in cells grown in medium containing 3 mg ml^{-1} glucose than in cells grown in 1 mg ml^{-1}. The addition of insulin did not increase the incorporation of label into collagen. We are now trying to determine whether the labelled carbon residues are in the polypeptide chain or in the disaccharide portion of collagen.

Rechler and Podskalny[18] have demonstrated insulin receptors in fibroblasts but many investigators cannot demonstrate insulin effects in cultured cells. Shaw and Amos[19] have shown that fibroblasts deprived of glucose become highly responsive to insulin; however, cells growing at ≥1 mg ml^{-1} glucose do not respond to insulin by increased glucose uptake. At these glucose concentrations therefore, glucose transport into the cells is gradient dependent and insulin independent. In our experiments the addition of insulin did not cause an additive effect on collagen secretion.

These data suggest that the ambient glucose concentration of fibroblasts can influence the amount, the type, and possibly the glycosylation of collagen secreted by these cells. The implications of this are of great importance to the diabetic patient. Recently, a highly glycosylated haemoglobin molecule, HbA$_{1C}$, has been found in increased amounts in poorly controlled diabetics[20]. Basement membranes isolated from kidney glomeruli of diabetic patients show a marked increase in the carbohydrate components linked to hydroxylysine of the collagenous protein[21]. The offspring of the diabetic mother are also exposed to hyperglycaemia and these infants are born with a variety of defects in development[22]. The ubiquitous presence of collagen throughout the body, its prime role as an inducer in early embryogenesis[23,24], and its susceptibility to structural changes have encouraged us to pursue further the regulation of synthesis and secretion of this molecule in the developing human organism.

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Spontaneous and induced cell-mediated reactivity to syngeneic cells

IN general, the immune system does not react with self-antigens to produce disease. Potential self-reactive clones are known to exist, however, and are thought to be controlled by thymic-derived suppressor lymphocytes (suppressor T cells). We have investigated the possibility that self-reactive clones of cells are expressed in B/W mice, and that such clones in other inbred mouse strains might become expressed after immunisation with specific antigens of the group A streptococcus. Our studies show that spleen cells from B/W mice respond spontaneously to syngeneic heart cells and to syngeneic kidney cells. In contrast, spleen cells from another inbred strain of mice, the BALB/c, do not respond spontaneously to syngeneic cells. A response to syngeneic cardiac determinants can, however, be induced in the BALB/c mouse by immunisation with cell membranes of group A streptococci.

Many observations, in both human and animal populations, suggest that at times self-reactivity (autoimmunity) does occur. One animal model of autoimmunity is the New Zealand Black/White hybrid mouse (B/W) (refs 1–3). In B/W mice, an imbalance of immune regulation precedes the onset of clinical autoimmune disease. Although the immunobiological events that result in autoimmune phenomena and the mechanisms by which these phenomena lead to pathological changes remain obscure, it has been proposed that the behaviour of T cells is critical to the development of autoimmunity[4–9]. T-cell suppressor function seems to be lost in the older B/W mouse, since exaggerated antibody responses to various antigens, such as type III pneumococcal polysaccharide and polyinosinic-polycytidylic acid, occur[5–9]. Further evidence for the loss of suppressor T-cell function is suggested by the decrease of concanavalin A-induced suppressor function in ageing mice[10]. Other T-cell functions also seem to be lost, since lymphoid cells from older B/W mice do not mount a graft against host response when transferred into allogeneic recipients.

Repeated exposure to antigens that cross react with self-determinants may induce an immune response that eventually results in autoimmunity. Repeated infections with group A streptococci are thought to be an essential factor in the pathogenesis of acute rheumatic fever[11]. Immunological cross reactions between streptococcal and mammalian antigens have been demonstrated, and these cross reactions seem to be mediated by antibodies specific for antigens of the streptococcal cell surface[12–15]. In addition, there are several reports which suggest that cell-mediated cross reactions between streptococcal and mammalian antigens also may occur[16–18].

In our experiments we used primary cultures of syngeneic heart, liver or kidney prepared from neonatal B/W or BALB/c mice. The hearts, livers and kidneys from sex-matched neonatal mice were removed, minced in Hank's calcium and magnesium-free balanced salt solution, trypsinised, treated with mitomycin-C to block DNA synthesis, and resuspended in Eagle's minimal essential medium supplemented with 10% heat-inactivated foetal calf serum. These cell suspensions served as stimulator cells. Responder cells were obtained from the spleens of mice 2–12 months of age. Several groups of BALB/c mice were first immunised with suspensions of group A streptococcal cell membranes. Spleen cells were prepared as single-cell suspensions in the medium described. Responder spleen cells (1.8×10^5) and stimulator cells (1.8×10^4) from heart, kidney or liver were mixed and incubated for 6 d. On the sixth day, all cultures received 2 μCi of tritiated thymidine, were incubated for an additional 24 h, and the amount of radioactive label incorporated into the acid-insoluble fraction of the cells was determined. Measurements of triplicate samples are reported as a stimulation index (SI)± standard error of the mean (s.e.m.); the SI is the ratio of the mean d.p.m. of responder cells in stimulated culture minus the d p m. of stimulator cells alone to the mean d.p.m. of responder cells alone.

The response of the responder spleen cells after incubation with syngeneic or with allogeneic stimulator cells is shown in Table 1. B/W spleen cells responded to syngeneic heart and syngeneic kidney cells as well as to BALB/c heart cells, but did not respond to syngeneic liver cells. On the other hand, BALB/c spleen cells from unimmunised animals did not respond either to syngeneic heart or kidney cells or to heart cells from the histocompatible B/W mice. Sets of BALB/c mice were inoculated with group A streptococcal cell membranes, and their spleen cells were shown to respond to stimulation by streptococcal cell membranes Spleen cells from these immunised BALB/c mice strongly reacted to both BALB/c heart cells and B/W heart cells, but did not respond to syngeneic kidney cells. The responsiveness of spleen cells from older B/W mice to syngeneic heart and syngeneic kidney cells persists, although they no longer respond to phytohaemagglutinin or concanavalin A (unpublished).

These findings demonstrate the existence of clones of cells in the B/W mouse that are spontaneously capable of responding to syngeneic cardiac and renal determinants. In addition, a similar response to syngeneic cardiac determinants can be induced in the BALB/c mouse by immunisation with cell membranes of group A streptococci. The response in the B/W mouse is present in young mice (1–2

Table 1 Response of spleen cells to syngeneic or allogeneic cell populations

Responder cells	Stimulator cells	SI±s.e.m.
B/W	B/W Heart	38.7±4.7
	B/W Kidney	9.5±0.2
	B/W Liver	1.3±0.4
	BALB/c Heart	92.2±7.1
BALB/c (unimmunised)*	BALB/c Heart	0.6±0.0
	BALB/c Kidney	0.0±2.9%†
	B/W Heart	0.0±1.3%†
BALB/c (immunised)‡	BALB/c Heart	82.0±0.3
	BALB/c Kidney	1 8±0.1
	B/W Heart	42.2±1.2

*The unimmunised BALB/c responder cells cultured with streptococcal cell membranes yielded an SI of 1.2±0.1, whereas the immunised BALB/c responder cells yielded an SI of 25.7±1.9 when cultured with the same cell membrane preparation.

†Responder cultures displayed less incorporation of ^{3}H-thymidine than control cultures of stimulator cells alone. SEM is reported as a percentage of the average of triplicate samples.

‡Immunised with group A type 14 streptococcal cell membranes.

months old) as well as in older mice (10–12 months old). These data are consistent with studies suggesting that the disturbance of immune regulation in the B/W mouse may include an early loss of suppressor T-cell function. A loss of suppressor T-cell function may well set the stage for the onset of autoimmune disease. Although these findings may shed some light on the pathogenesis of autoimmune disease in general, as well as that of rheumatic carditis, the observations that cell-mediated reactivity to syngeneic cardiac cells can occur spontaneously or can be induced by microbial antigens may be of broad immunobiological significance.

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Inhibition of platelet aggregation by a placental substance with prostacyclin-like activity

The demonstration that certain arteries and veins have the ability to generate from prostaglandin (PG) endoperoxides an unstable substance called prostacyclin (PGI$_2$) (ref. 1), which will inhibit platelet aggregation[2,3], should lead to a reappraisal of the homeostatic mechanisms preventing thrombosis. It has been suggested that the endothelium of these blood vessels contains an enzyme which is capable of converting PG endoperoxides from circulating platelets into an unstable substance, prostacyclin. This inhibits platelet aggregation and so prevents the initial step of thrombus formation within the vessel[4]. Damage to the endothelium of the vessel wall may prevent production of the enzyme PGI$_2$ synthetase, and the subsequent reduction in the local synthesis of prostacyclin could facilitate platelet aggregation in the area of damage. Maintenance of an adequate circulation within the placenta is critical to foetal well-being. This circulation is one both of low pressure and velocity and the presence of a substance which has a potent inhibitory effect on platelet aggregation would seem desirable for its maintenance. We demonstrate here the ability of normal placental tissue to generate a labile substance which has the ability to inhibit ADP-induced platelet aggregation. The properties exhibited by this substance are suggestive of it being PGI$_2$.

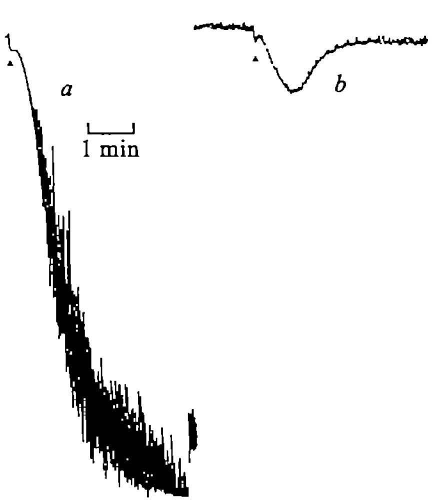

Fig. 1 Aggregation of platelet rich plasma (PRP) pre-incubated with placental tissue. PRP was prepared from citrated plasma by centrifugation at 2,000 r.p.m. for 10 min. Aggregation was induced by addition of ADP to a concentration of 5 µm (▲). *a*, The control response of 0.9 ml PRP to ADP. *b*, The effect of pre-incubation of 0.9 ml PRP with 10 mg washed placental tissue for 20 min at 22 °C before addition of ADP.

Venous blood was collected from the same subject who had not taken prostaglandin synthetase inhibitors for at least 2 weeks. Citrate solution 3.1% (9 : 1, v/v) was used as the anticoagulant and platelet rich plasma (PRP) and platelet poor plasma were prepared by centrifugation. Platelet aggregation was carried out as previously described[5], using ADP in a final concentration of 5 μM.

Pieces of placental tissue (10–40 mg) were taken at random from the maternal surface immediately after delivery. In all cases the pregnancies and deliveries were entirely normal. The placental pieces were washed several times to remove blood and kept in Krebs solution gassed with 95% O$_2$: 5% CO$_2$ until use.

Pre-incubation of PRP with placental tissue inhibited subsequent ADP-induced platelet aggregation (Fig. 1). Incubation of placental tissue in Tris buffer and the subsequent addition of an aliquot of the supernatant to PRP quantitatively inhibited ADP-induced platelet aggregation. Heating of this supernatant at 37 °C for 10 min or boiling for 0.25 min abolished its anti-aggregatory properties (Fig. 2). Platelets aggregated by previous addition of ADP could be disaggregated by the addition of an aliquot of supernatant derived from incubation of placental tissue in Tris buffer (Fig. 3).

Pretreatment of placental tissue with indomethacin did not affect its anti-aggregatory properties when subsequently incubated with PRP, whereas pretreatment with tranylcypromine abolished the anti-aggregatory activity (Fig. 4).

These experiments show that placental tissue can spontaneously generate a substance which inhibits platelet

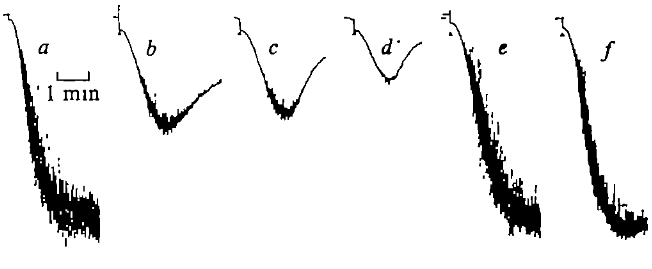

Fig. 2 Effects on platelet aggregation of supernatant from placental tissue incubated in buffer. 40 mg of placental tissue was incubated in 500 µl 0.05 M Tris, *p*H 7.5 for 20 min at 22 °C. The supernatant was added in varying amounts. *a*, 0 µl; *b*, 25 µl; *c*, 50 µl and *d*, 100 µl—each added to 0.9 ml PRP and incubated for 1 min at 37 °C before addition of ADP 5 µM. The same experiments were carried out using 100 µl of supernatant which had been boiled for 0.25 min (*e*) or heated at 37 °C for 10 min (*f*).

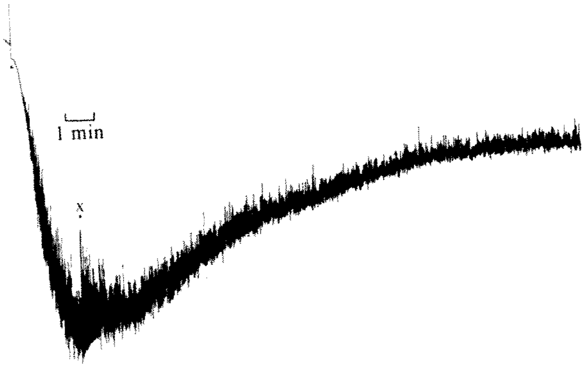

Fig. 3 Reversal of ADP-induced platelet aggregation by supernatant from placental tissue incubated in buffer. 0.9 ml PRP was aggregated by addition of ADP (▲) and reversal of aggregation caused by addition at (X) of 100 μl supernatant from 20 mg placental tissue incubated in 200 μl 0.05 M Tris, pH 7.5 for 20 min at 22 °C.

aggregation. Addition of placental tissue itself (Fig. 1) or supernatant from tissue incubated in buffer at room temperature (Fig. 2) to PRP causes significant inhibition of ADP-induced platelet aggregation. Abolition of the anti-aggregatory activity by heating at 37 °C for 10 min or boiling for 0.25 min (Fig. 2) is in agreement with observations on the instability of prostacyclin[3]. Our findings that supernatant from placental tissue incubated in buffer can disperse preformed platelet aggregates (Fig. 3) is in agreement with the findings of other workers[6,7], confirming that this unstable substance affects platelet aggregation by means other than direct competition with thromboxane A_2 (ref. 8).

Pretreatment of the tissue with indomethacin, an inhibitor of the cyclo-oxygenase enzyme[9] prevents formation in the tissue of PG endoperoxides and primary PGs known to inhibit platelet aggregation. Incubation of this indomethacin-treated tissue wih PRP still inibits platelet aggregation (Fig. 4). This suggests that the enzyme PGI_2 synthetase which may be present in the placenta is unaffected by indomethacin and can act on platelet endoperoxides to produce prostacyclin.

Pretreatment of placental tissue with tranylcypromine, a known inhibitor of PGI_2 synthetase[4] does abolish the inhibition of platelet aggregation seen when placental tissue is incubated with PRP (Fig. 4).

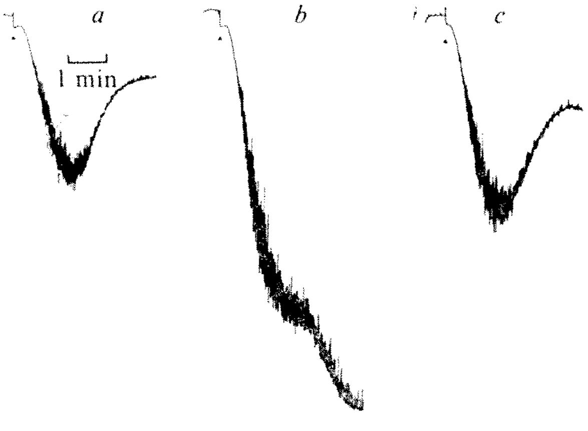

Fig. 4 Treatment of placental tissue with inhibitors of enzymes in the prostaglandin synthetic pathway. 20 mg of placental tissue was incubated for 20 min at 22 °C in a, 200 μl 0.05 M Tris, pH 7.5; b, 200 μl tranylcypromine (50 μg ml^{-1} in 0.05 M Tris, pH 7.5); c, 200 μl indomethacin (1 μg ml^{-1} in 0.05 M Tris, pH 7.5.). The tissue was removed, washed three times in Tris buffer before incubation with 0.9 ml PRP for 15 min at 22 °C and subsequent addition of ADP (▲).

Thus placental tissue when in contact with platelets seems to generate an unstable substance, inhibiting platelet aggregation and exhibiting the previously described properties of prostacyclin. The generation of this substance could be an important factor in the maintenance of placental circulation. Localisation and measurement of the PGI_2 synthetase capacity, which these experiments suggest might be present in the placenta, is the next step in understanding the homeostasis of the placental circulation. The decreased rate of platelet disaggregation in the plasma of patients suffering from placental insufficiency[10] is suggestive of decreased prostacyclin levels or PGI_2 synthetase capacity.

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Prostacyclin is the major prostaglandin released from the isolated perfused rabbit and rat heart

PROSTACYCLIN (prostaglandin X, PGI_2), a newly discovered prostaglandin[1-3], is formed by microsomes from several organs, such as pig and rabbit aorta[2] and rat stomach[4], and by fresh human arterial and venous tissue incubated with PGH_2 (ref. 5). As inhibitor of blood platelet aggregation, it is 30 times more potent than PGE_1 (ref. 2) and could thus help in preventing the formation of intra-arterial thrombi. It has been suggested[1] that the endoperoxides released by the platelets can be converted into prostacyclin by vascular tissue. Prostacyclin is chemically unstable in aqueous solution: it hydrolyses to 6-oxo-$PGF_{1\alpha}$ (refs 3, 4; Fig. 1) which does not inhibit platelet aggregation[2]. We report that isolated perfused rabbit and rat hearts produce a labile factor which strongly inhibits blood platelet aggregation; the release of this substance is abolished by indomethacin. Using a physicochemical method, we found 6-oxo-$PGF_{1\alpha}$ in substantial amounts in the perfusates. These findings indicate that prostacyclin is the major prostaglandin released from the heart.

Hearts were perfused with a Krebs–Henseleit buffer solution according to Langendorff or as modified by De Deckere and Ten Hoor[12]. The latter method is based on the observation that in the isolated perfused heart a small part (2–5%) of the perfusion fluid reaches the surface of the heart through the interstitial space and the lymphatics. This smaller flow is separated from the main flow by tying off the veins of the right and left atrium and cannulating the pulmonary artery. Most of the perfusion fluid is ejected by the right ventricle through the cannula (Q_{rv}), a small amount passes the interstitium and drips from the heart (Q_i). As the flow rate of Q_i is small, the concentrations of substances released into Q_i are relatively high.

Prostacyclin-like activity was determined direct in Q_i by measuring the inhibition of ADP-induced rat platelet aggregation (Fig. 2)[6]. The biological activity was standardised against PGE_1. As rat platelets are insensitive to PGD_2 and PGE_2,

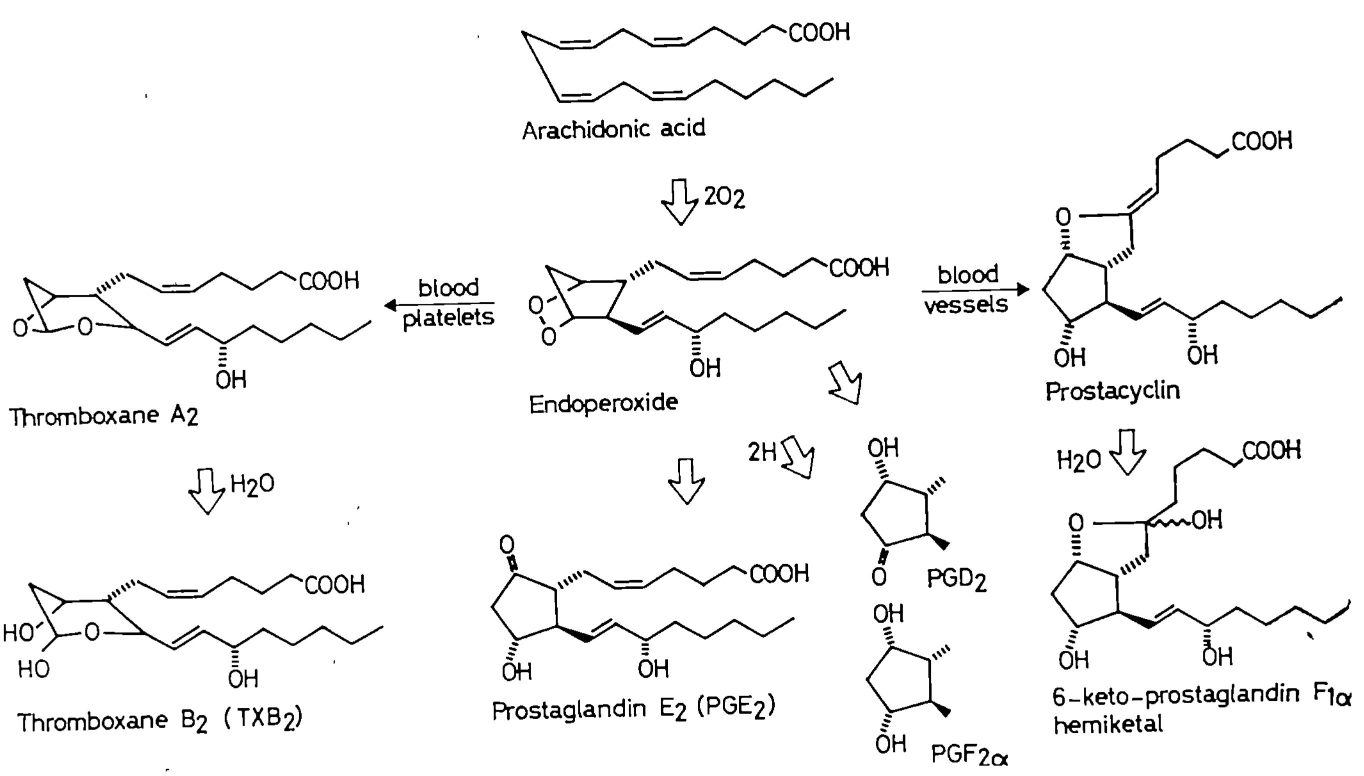

Fig. 1 Routes in the metabolism of prostaglandin-endoperoxide.

interference from 'stable' prostaglandins in Q_1 cannot be expected. PGE_2-like activity was assayed on the isolated gerbil colon[7] in ethyl acetate extracts of acidified perfusates. Antagonists were used as described previously[8]. PGE_2, PGD_2, $PGF_{2\alpha}$, TXB_2 (thromboxane B_2) and 6-oxo-$PGF_{1\alpha}$ (Fig. 1) were extracted, purified by thin-layer chromatography as their methoximated pentafluorobenzyl esters and, after silylation, determined by gas chromatography with electron-capture detection[9] (Table 1). Figure 3 gives examples of the results of the determination of PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$.

The isolated perfused rabbit heart released at least twice as much 6-oxo-$PGF_{1\alpha}$ as PGE_2, the amount (8–12 ng per min per g dry weight) being more than that of all other prostaglandins together (Table 2). The results of the bioassay on the gerbil colon and the gas-chromatographic determinations of PGE_2 correlate reasonably well. Since 6-oxo-$PGF_{1\alpha}$ is the stable hydrolysis product from prostacyclin, it is most probably prostacyclin that was released from the isolated rabbit heart. Freshly collected Q_I showed a distinct inhibition of platelet aggregation (Fig. 2 and Table 3), an ability which disappeared after incubating Q_I for 10–15 min at 37 °C. Perfusion of the heart with indomethacin (1 μg ml^{-1}) completely abolished the anti-aggregatory activity of the perfusate; anoxia for 6 min increased this activity only moderately. Because of the much greater dilution, no inhibition of platelet aggregation could be detected with Q_{IV}, although in this perfusate 6-oxo-$PGF_{1\alpha}$ was present according to gas chromatography.

Perfused rat hearts also released 6-oxo-$PGF_{1\alpha}$ (Table 3) as the major prostaglandin, but anoxia did not increase prostaglandin output. In ten rat hearts Q_I was collected for 5 min

Table 1 R_f values of the methoximated (MO) pentafluorobenzyl (PFB) esters after TLC and retention times (R_t) of the trimethylsilylated (TMS) derivatives during GLC

Compound*	%†	R_f (MO·PFB)	R_t‡ (MO·PFB·TMS) (min)	Compound*	%†	R_f (MO·PFB)	R_t‡ (MO·PFB·TMS) (min)
PGD_2	90	0.69	16.0	Thromboxane B_2	43	0.46	19.8
	10	0.57	14.7	(TXB_2)	57	0.38	19.8
PGE_2	74	0.54	17.5	6-oxo-$PGF_{1\alpha}$	58	0.37	22.0
	26	0.46	15.2		42	0.31	22.2
ω-nor-PGE_2	68	0.53	14.2	$PGF_{2\alpha}$	—	0.28	15.2
	32	0.45	12.3	$PGF_{1\alpha}$	—	0.25	17.8

100 ng ω-nor-PGE_2 (ref. 10) and 100 ng $PGF_{1\alpha}$ in 1 ml methanol were added to the perfusates as internal standards. (For the experiment of Table 2, the two standards were added after extraction) The solution was then acidified with HCl (6 mol l^{-1}), drop by drop, until *p*H 4.0. The perfusates were extracted twice with equal volumes of ethyl acetate. The extract was taken to dryness *in vacuo* at 30 °C and the residue rinsed with 2–6 ml methanol into a small test tube. The solvent was removed with nitrogen, and 0.2 ml 2% methoxyamine HCl in pyridine was added. After 16 h at 20 °C, the greater part of the pyridine was removed with nitrogen; 0.5 ml H_2O was added and the prostaglandins were extracted with 2×2 ml ether. To the residue of this extraction, 30 μl pentafluorobenzylbromide was added followed by 60 μl 10% diisopropylethylamine in acetonitrile[9]. After standing for 1 h at 20 °C, the solvents were again removed with a nitrogen stream. The residue was applied as a 2-cm band on a 0.25-mm thick silica gel Fertigplatte (Merck) and developed with chloroform–methanol (90:6, v/v). The appropriate bands were scraped off the plate and eluted with 1–2 ml ether–methanol (2:1, v/v). The eluates were evaporated, the residue was silylated with 25 μl *bis*-(trimethylsilyl)-trifluoroacetamide–pyridine (1:1, v/v) for 1 h at 20 °C and excess reagent removed with nitrogen. Between 0.5 and 5 ng of the PFB esters was injected for gas chromatography, giving sufficiently large peaks with the electron-capture detector.

*Authentic prostaglandins used as references and standards were obtained by biosynthesis from the corresponding unsaturated fatty acids or by incubating PGH_2 with blood platelets (TXB_2) or with sheep aorta microsomes (6-oxo-$PGF_{1\alpha}$).

†The methoximes were present as two isomers (*syn* and *anti*) in the ratio as indicated. $PGF_{2\alpha}$ and $PGF_{1\alpha}$ cannot give a methoxime.

‡2% SE 30, $l = 150$ cm, $d = 0.2$ cm, at 230 °C; carrier gas: argon–10% methane, 25 ml min^{-1}. A Hewlett–Packard 5700 A gas chromatograph with a linear electron capture detector 18713 A was used.

Table 2 Prostaglandin release (ng per min per g dry weight) from the isolated, perfused rabbit heart after anoxia

Group	Gerbil colon	Gas chromatography				
	PGE_2-like activity	PGE_2	PGD_2	$PGF_{2\alpha}$	TXB_2	6-oxo-$PGF_{1\alpha}$
1 (n=8)	4.7±0.6	3.5	0.9	1.8	2.2	9.3
2 (n=8)	3.3±0.5	3.9	1.2	1.8	2.1	9.9

In two separate experiments, eight rabbit hearts (about 0.7 g dry weight) were perfused according to Langendorff (for details, see Table 3). Fifteen minutes after starting the perfusion, the hearts were perfused with an oxygen-free buffer for 3 min. The buffer was then reoxygenated and the perfusion fluid collected for 10 min to give about 400 ml perfusate per heart. This fluid, after acidification, was extracted with ethyl acetate (Table 1). Aliquots of the extracts were tested individually for PGE_2-like activity; PGE_2 served as standard. The other prostaglandins found in the perfusate have relatively little activity on the gerbil colon. The remaining parts of the samples were pooled in the two groups of eight and the five prostaglandins determined in triplicate.

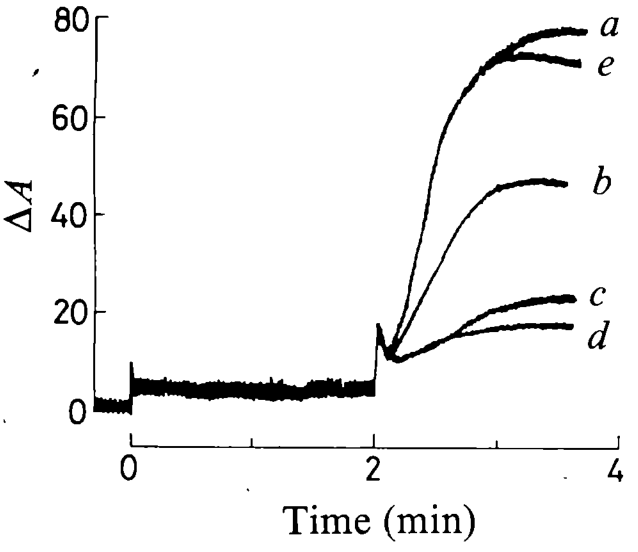

Fig. 2 Inhibition of ADP-induced rat platelet aggregation by freshly collected interstitial effluent Q_i from the isolated perfused heart. Changes in absorbance (ΔA) of rat platelet-rich plasma (PRP; final volume 3 ml) in a modified Vitatron photometer at 37 °C. At $t = 0$, 0.2 ml buffer solution (Krebs–Henseleit) (*a*), 0.2 ml buffer solution with a PGE_1 concentration of 60 (*b*) or 120 ng ml^{-1} (*c*), 0.2 ml of Q_i (*d*) or 0.2 ml of Q_i heated at 37 °C for 15 min (*e*) was incubated with PRP for 2 min ADP (final concentration 94 ng ml^{-1}) was then added to induce aggregation. Tracings have been superimposed. As prostacyclin is assumed to be 30 times more potent than PGE_1 (ref. 2), it can be calculated that in this case Q_i contains 4.5 ng ml^{-1} prostacyclin. Tracing *e* shows that heating of Q_i abolishes its anti-aggregatory activity.

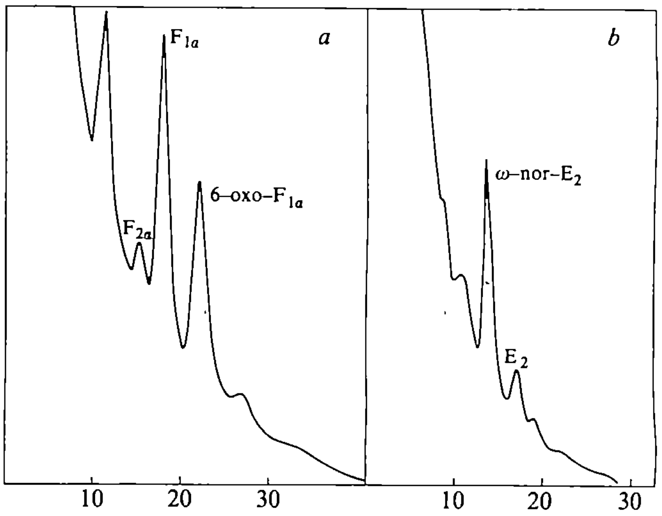

Fig. 3 Gas-chromatographic results of the determination of $PGF_{2\alpha}$ (*a*), 6-oxo-$PGF_{1\alpha}$ (*a*) and PGE_2 (*b*) in rabbit heart perfusates. The methoxime-pentafluorobenzylester-trimethysilylether derivatives were used for the electron-capture detection. $PGF_{1\alpha}$ (100 ng) and ω-nor-PGE_2 (100 ng) had been added as internal standards. Attenuation × 128. For experimental details, see Table 1.

(from 20 to 25 min after starting the perfusion). The prostacyclin-like activity was determined by the platelet aggregation test, the prostacyclin production being 3.3±0.4 ng per min per g dry weight. In four of ten hearts, the prostacyclin release, observed for 1 h at 15-min intervals, was nearly constant. As the release of prostacyclin into Q_i and Q_{rv} is about the same, the total release of prostacyclin from the rat heart can be estimated from the amount released into Q_i.

Our results show that prostacyclin is the major prostaglandin released from perfused rabbit and rat hearts. The negligible amount of thromboxane B_2 found in the perfusion fluid renders it likely that there are hardly any blood platelets present in the isolated perfused heart. This indicates that the heart can synthesise prostacyclin direct from arachidonic acid. We also found that the amount of prostacyclin released from the heart is almost constant during perfusion for more than 1 h, and that it is abolished by infusion of indomethacin (1 μg ml^{-1}). Moncada and co-workers found that in rabbit and pig aortas[1] and in human arteries and veins[5] prostacyclin is almost exclusively synthesised from prostaglandin endoperoxides. They suggest that *in vivo* endoperoxides originating from blood platelets, in active contact with the endothelial layer, are converted into prostacyclin. Our results show that the rabbit and rat heart can synthesise prostacyclin in the absence of blood platelets.

The physiological consequences of these findings cannot yet

Table 3 Prostacyclin and prostaglandin release (ng per min per g dry weight) from the isolated perfused rabbit and rat heart

	Rabbit			Rat				
	Perfusate	Prostacyclin	6-oxo-$PGF_{1\alpha}$	PGE_2	PGD_2	$PGF_{2\alpha}$	TXB_2	6-oxo-$PGF_{1\alpha}$
Before anoxia	Q_{rv}	—	2.7	2.9	<0.5	0.5	0.6	2.2
	Q_i	4.2	4.0	0.5	<1	0.4	0.3	3.1
Following anoxia	Q_{rv}	—	2.2	1.2	n.d.*	0.6	n.d.*	1.8
	Q_i	6.2	8.7	0.7	n.d.*	0.2	n.d.*	1.7

Hearts were perfused with a Krebs–Henseleit buffer solution, gassed with O_2–CO_2 (95:5) and supplemented with glucose (11.1 mmol l^{-1}) and EDTA (0.5 mmol l^{-1}), by the modified Langendorff technique of De Deckere and Ten Hoor[11]. Perfusion pressure was 60 mmHg for rabbit hearts, 80 mmHg for rat hearts; stimulation frequencies were 250 and 360 min^{-1}. Twenty minutes after starting the perfusion Q_{rv} and Q_i were collected for 20 min from two rabbit hearts (about 1 g dry weight per heart; hearts were dried at 80 °C for 24 h; dry weight was about 20% of wet weight) and four rat hearts (about 0.2 g dry weight per heart) to give a total of 1400 ml Q_{rv} and 22 ml Q_i from the rabbit hearts and 1,200 ml Q_{rv} and 30 ml Q_i from the rat hearts. Next, the rabbit hearts were perfused with a buffer, gassed with N_2–CO_2 (95:5) for 6 min, the rat hearts for 5 min. Following this anoxic period, the perfusion fluid was reoxygenated and Q_{rv} (rabbit 850 ml, rat 600 ml) and Q_i (rabbit 40 ml, rat 20 ml) were collected for 10 min. Prostacyclin-like activity was determined direct in fresh Q_i samples collected on ice under N_2–CO_2 (95:5), using the blood platelet aggregation assay[6] and assuming an activity of 30 times that of PGE_1 (ref. 2). Prostaglandins were determined by gas chromatography.

*n.d., Not determined.

be estimated. The continuous production of prostacyclin by the heart and the anti-aggregatory and vasodilatory activity suggest that the main function of prostacyclin production is to protect the coronary circulation against the formation of blood platelet aggregates, thus preventing the occlusion of small branches of the vulnerable coronary tree. If the present results are extrapolated to the human heart, prostacyclin could be a key substance with respect to the origin[11] as well as the prevention of coronary diseases.

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Transitory expression of Thy-1 antigen in skeletal muscle development

A CELL surface differentiation antigen known as Thy-1 (formerly called theta[1]) is expressed during development of brain and thymocytes in both rat and mouse (see ref. 2 for review). Thy-1 is present in brain and on thymocytes throughout adult life, as well as on a subpopulation of peripheral lymphocytes. Both rat and mouse fibroblasts, and some fibroblastic cell lines, express Thy-1 *in vitro*[3] and mouse Thy-1 has been reported on epidermal cells (see ref. 2 for review) and a mammary tumour[4]. The Thy-1 antigen has been reported lacking in other adult tissues, including muscle[1]. We present here evidence for an antigen with the serological specificity of Thy-1 on rat myogenic cell lines and embryonic rat muscle differentiating *in vitro*. The presence of the antigen was transitory, being expressed on myoblasts and newly developed myotubes, but disappearing as myotubes differentiated.

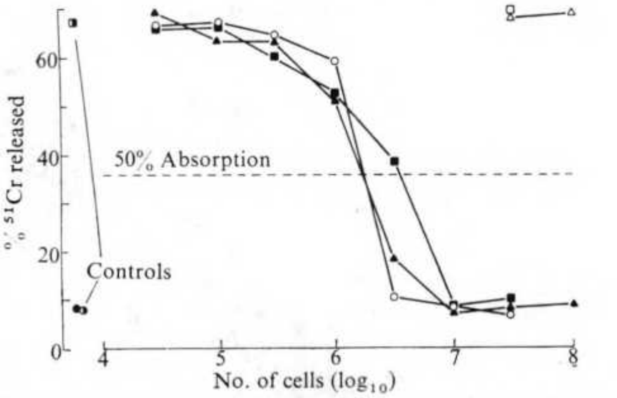

Fig. 1 Exponentially growing cells were harvested in HEPES buffered medium containing 2% FCS (ABM/FCS). L6 and M3A were loosened from the dishes before cell collection by incubation at 37 °C for 5 min in phosphate buffered saline (PBS) lacking Ca^{2+} and Mg^{2+} and containing 2 mM EDTA. Graded numbers of cells were added to ATS (0.2 ml of a 1:80 dilution in HBM/FCS) for 45 min at 4 °C, then removed by centrifugation. Residual antibody was assayed by adding 0.1 ml of absorbed ATS to ^{51}Cr-labelled SIA target cells (2×10^5) in 0.2 ml HBM/FCS containing fresh rat serum complement at a dilution of 1:8. After 45 min at 37 °C, 2 ml HBM/FCS was added, the tubes were centrifuged for 10 min at 475 g, and supernates and pellets were counted separately. %^{51}Cr release was calculated as follows

$$\frac{\text{c.p.m. in supernate}}{\text{c.p.m. in supernate}+\text{c.p.m. in pellet}}\times 100$$

Cell lines used for absorption were: ○, SIA; ▲ C58[NT]D; ■, L6; △, S194; □, M3A. Controls were ^{51}Cr SIA target cells: ●, alone (spontaneous lysis); ○, with rat complement; □, with rat complement + unabsorbed ATS at 1:80.

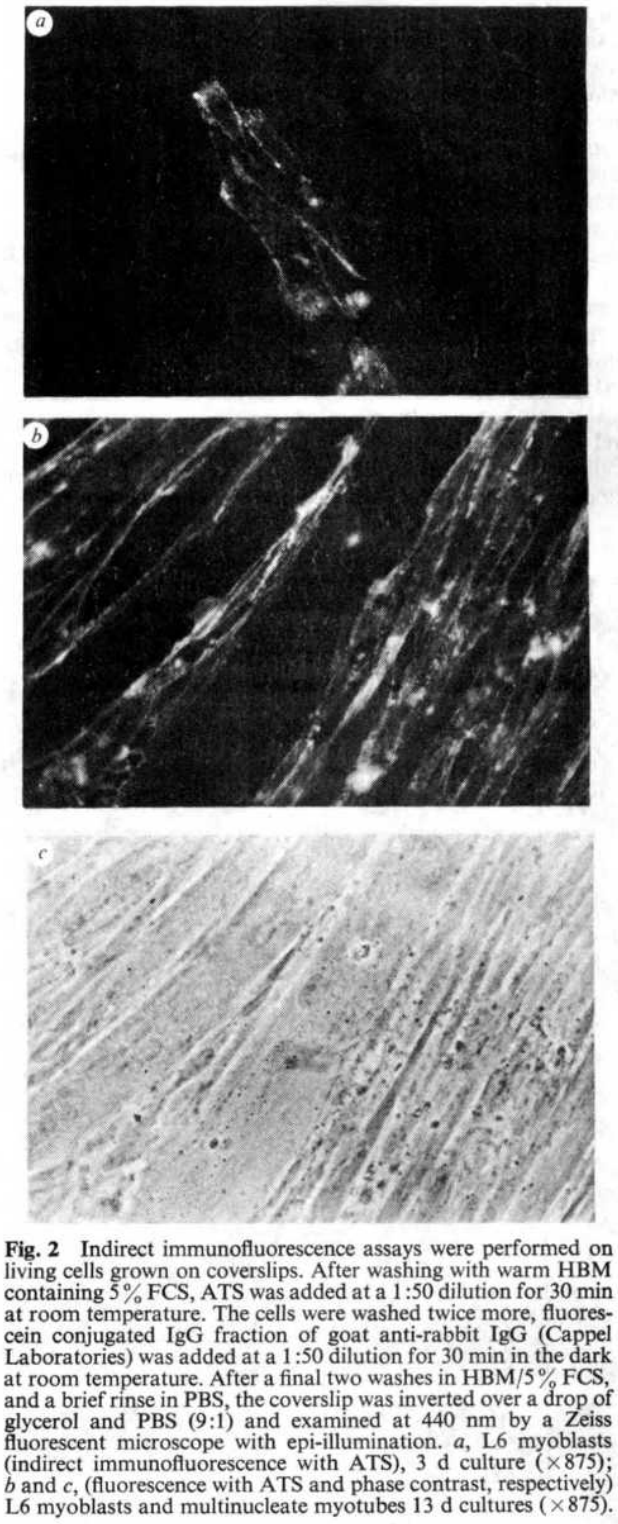

Fig. 2 Indirect immunofluorescence assays were performed on living cells grown on coverslips. After washing with warm HBM containing 5% FCS, ATS was added at a 1:50 dilution for 30 min at room temperature. The cells were washed twice more, fluorescein conjugated IgG fraction of goat anti-rabbit IgG (Cappel Laboratories) was added at a 1:50 dilution for 30 min in the dark at room temperature. After a final two washes in HBM/5% FCS, and a brief rinse in PBS, the coverslip was inverted over a drop of glycerol and PBS (9:1) and examined at 440 nm by a Zeiss fluorescent microscope with epi-illumination. *a*, L6 myoblasts (indirect immunofluorescence with ATS), 3 d culture (×875); *b* and *c*, (fluorescence with ATS and phase contrast, respectively) L6 myoblasts and multinucleate myotubes 13 d cultures (×875).

Rabbit anti-rat-thymocyte serum (ATS) was raised by injecting 10^9 Lewis rat thymocytes, intraperitoneally weekly together with *B. pertussis*, and intradermally every 6–12 weeks with complete Freund's adjuvant. Complement dependent cytotoxicity was titrated on rat thymocytes. Sera of high cytotoxic titres were pooled, heated at 56 °C and absorbed sequentially at 4 °C with rat red blood cells, liver and kidney. A similarly raised and absorbed antiserum was reported to have specificity for purified Thy-1 glycoprotein (see ref. 5). Full details of our pooled antiserum's specificity will be published elsewhere (J.F.L. and V.A.L., in preparation). Its Thy-1 specificity was established by the following criteria: (1) complement dependent cytotoxicity for rat and mouse Thy-1 bearing lymphocytes and lymphomas but not for mouse myelomas; (2) absorption of cytotoxicity against a mouse lymphoma by Thy-1 positive mouse lymphomas, but not by Thy-1 negative variants[6]; (3), absorption by rat brain and thymus, but not by liver, kidney, adult muscle or red blood cells; and (4), precipitation of the 25,000 molecular weight glycoprotein T25 (bearing Thy-1 allospecificity[7]) from ^{125}I-labelled proteins solubilised from rat and mouse thymocyte surfaces.

Thy-1 was assayed by quantitative absorption of cytotoxicity[8] and by indirect immunofluorescence. In assaying for residual antibody after absorption, mouse rather than rat Thy-1 bearing lymphomas were used as cytotoxic targets to ensure detection of a Thy-1 specificity since T25 was the only detectable molecule precipitated from mouse thymocytes by ATS.

Cell lines assayed included: cloned rat myogenic lines, L6 (derived by repeated subculture of foetal limb muscle[9]) and M3A (a non-fusing variant of L6 (ref. 10)), a W/Fu rat thymoma (C58[NT]D (ref. 11)), a BALB/c mouse lymphoma bearing Thy-1 (SIA (ref. 12)) and a BALB/c myeloma (S194 (ref. 12)). All cells grew in suspension in Falcon Petri dishes in Dulbecco's modified Eagles medium (DMEM) with 10% foetal calf serum (FCS), except L6 and M3A which grew attached to Falcon tissue culture dishes and were harvested with 2 mM EDTA for absorption assays.

Figure 1 illustrates the results of absorption assays. Cytotoxicity of ATS for the Thy-1 bearing target SIA was 50% absorbed by 1.5×10^6 rat or mouse thymoma cells. This is approximately twice the absorptive capacity of rat thymocytes. Fifty per cent cytotoxicity was absorbed by 3.0×10^6 L6 myoblasts. Thus the absorptive capacity of each L6 cell approximated that of a thymocyte. Estimation of cell surface area allowed calculation of the approximate antigen density represented by the absorption capacities (see ref. 8). When rounded up after harvesting the

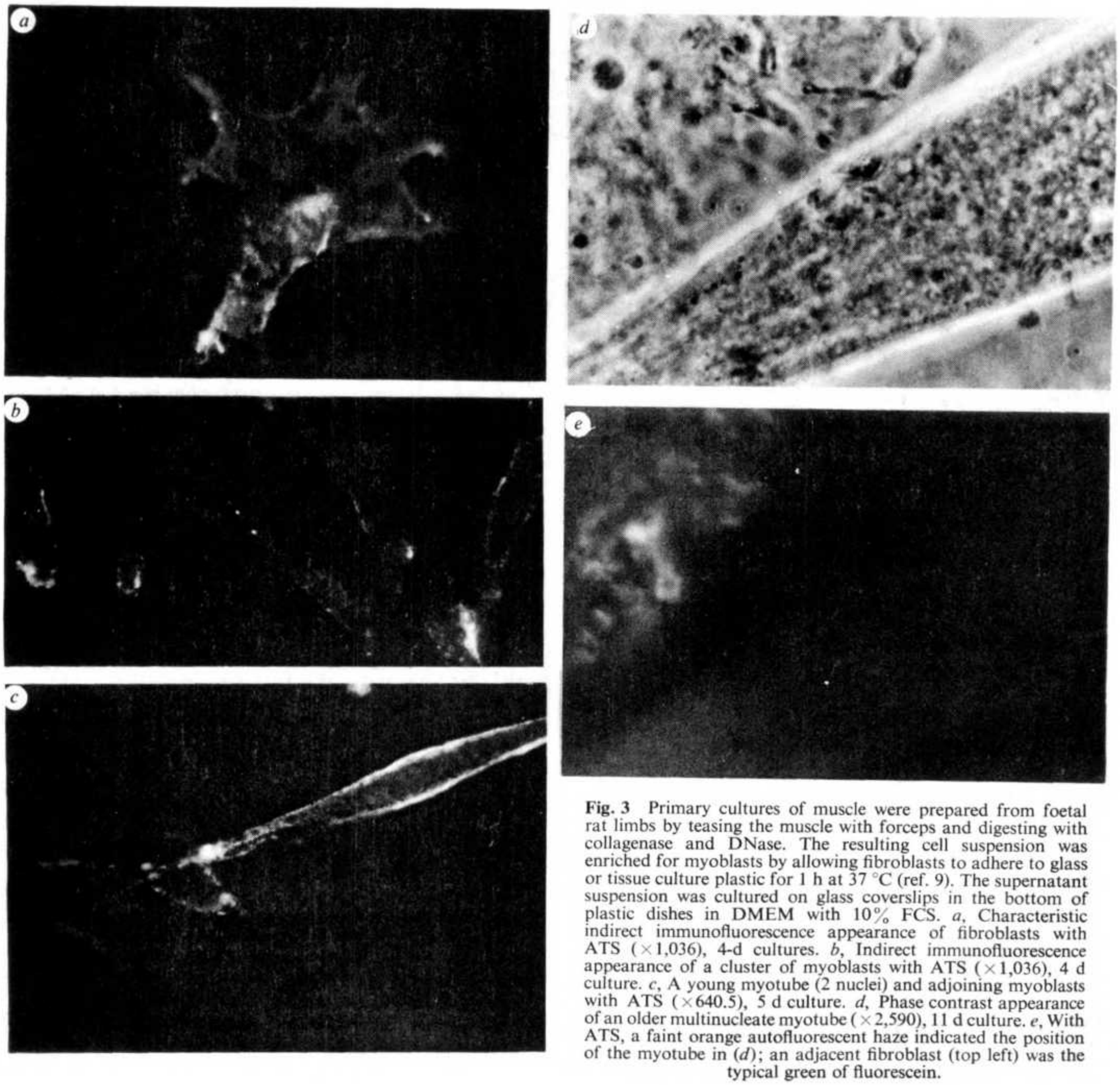

Fig. 3 Primary cultures of muscle were prepared from foetal rat limbs by teasing the muscle with forceps and digesting with collagenase and DNase. The resulting cell suspension was enriched for myoblasts by allowing fibroblasts to adhere to glass or tissue culture plastic for 1 h at 37 °C (ref. 9). The supernatant suspension was cultured on glass coverslips in the bottom of plastic dishes in DMEM with 10% FCS. *a*, Characteristic indirect immunofluorescence appearance of fibroblasts with ATS (×1,036), 4-d cultures. *b*, Indirect immunofluorescence appearance of a cluster of myoblasts with ATS (×1,036), 4 d culture. *c*, A young myotube (2 nuclei) and adjoining myoblasts with ATS (×640.5), 5 d culture. *d*, Phase contrast appearance of an older multinucleate myotube (×2,590), 11 d culture. *e*, With ATS, a faint orange autofluorescent haze indicated the position of the myotube in (*d*); an adjacent fibroblast (top left) was the typical green of fluorescein.

antigen density of the L6 myoblast was approximately one-third that of a thymocyte. M3A, its non-fusing variant, and S194, a myeloma, did not absorb ATS activity, indicating lack of Thy-1 antigen (less than 0.5–1 % of thymocytes).

As L6 cells mature, myoblasts fuse forming multinucleate myotubes which bear nicotinic acetylcholine receptors, are contractile, contain enzymes characteristic of mature muscle and are capable of forming functional cholinergic synapses when co-cultured with appropriate neurons[13]. We noticed that older L6 cultures containing many large myotubes (14 d after plating) absorbed less ATS cytotoxicity than cultures of recently plated myoblasts. The distribution of antigen on L6 was examined by indirect immunofluorescence with ATS (Fig. 2). With fine focus adjustment uniform speckling was observed over the surface of all myoblasts (Fig. 2*a*). L6 cells incubated with either ATS previously absorbed with rat brain or with fluoresceinated anti-rabbit IgG alone, were not fluorescent. In older cultures large, multinucleate L6 myotubes lacked surface fluorescence, in contrast to surrounding myoblasts (Fig. 2*b* and *c*).

Immunofluorescence studies of Thy-1 in cultured foetal muscle are complicated by the presence of fibroblasts, which express Thy-1 (ref. 3). The modified spindle shape of a typical myoblast (Fig. 3*b*) distinguishes it from flattened irregular-shaped fibroblasts (Fig. 3*a*). In early primary cultures all cells fluoresced with ATS, the pattern on primary myoblasts being similar to L6 myoblasts. The density of fluorescent speckles on individual primary myoblasts varied considerably, however. The brightest myoblasts resembled L6 in both pattern and intensity of fluorescence; others had fewer speckles distributed randomly over the cell surface. The surface fluorescence of newly formed myotubes, with just a few nuclei, appeared more dense and brighter than that of adjacent myoblasts (Fig. 3*c*). Fluorescence on myoblasts, young myotubes and fibroblasts was absorbed by rat brain, but not by adult muscle or liver. Mature multinucleate myotubes (Fig. 3*d*) were virtually invisible under blue light (Fig. 3*e*).

Further evidence for the Thy-1 specificity of immunofluorescence on cultured muscle cells includes the following: (1) Rat brain cell lines which were negative for Thy-1 by absorption assay failed to fluoresce with ATS while those positive by absorption were also positive by immunofluorescence (J.F.L. and V.A.L., in preparation). (2) The positive immunofluorescence of mouse primary muscle cultures with ATS, was absorbed by a Thy-1 positive lymphoma, but not by its Thy-1 negative variant[6]. (3) Primary rat myoblasts and fibroblasts were positive by indirect immunofluorescence using an antiserum raised against T25 (ref. 7), a glycoprotein bearing Thy-1 specificities.

Accompanying *in vitro* differentiation of foetal muscle and myogenic cell lines there was apparent loss of Thy-1 which was expressed on myoblasts but was not detectable by immunofluorescence on mature myotubes. This observation is consistent with lack of Thy-1 in adult muscle. The transitory appearance of Thy-1 on myogenic cells at the time of fusion and its absence on a non-fusing variant of the L6 myoblast line suggests that this antigen may be functional in normal muscle development. Glycoproteins bearing Thy-1 antigenic determinants have been purified from rat brain and thymus[2]. The chemical nature of Thy-1 antigen expressed in developing muscle is yet to be established.

The occurrence on myoblasts and immature myotubes of an antigen characteristic of thymocytes represents another unexplained association between muscle and thymus. Others include: (1) the presence of striated muscle ('myoid') cells as a constituent of normal mammalian, avian and reptilian thymuses[14]; (2) the presence in rat thymus of nicotinic acetylcholine receptors characteristic of muscle[15]; (3) the concurrence of pathological lesions in thymus and muscle in at least two spontaneous disease processes—myasthenia gravis and murine muscular dystrophy[16]; (4) the reported *in vivo* effect on neuromuscular transmission of thymopoietin, a putative thymic hormone which induces the expression of Thy-1 on cultured prothymocytes[17,18]. The possible function of an antigen shared by developing muscle and thymocytes, T lymphocytes and brain is open to speculation.

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Relationship between Na:K and Na:Na exchange by the sodium pump of skeletal muscle

THE sodium pump can catalyse several modes of cation exchange (for example Na:K, Na:Na, and K:K), which have been characterised mainly in human red blood cells[1-3], squid giant axon[4-6], and frog skeletal muscle[7-9]. In all three tissues it seems that intracellular ADP levels determine the extent to which the pump can engage in Na:Na exchange[2,6,9,10]. Yet, despite its obvious kinetic implications, the basic question of how the 'normal' Na:K exchange is affected by the appearance of ADP-induced Na:Na exchange, has not been answered. Here we present evidence that, in frog skeletal muscle, elevated intracellular ADP levels have little effect on pre-existing electrogenic Na:K exchange, but cause Na:Na exchange to appear in addition to ongoing Na:K exchange.

Frog sartorius muscles, which had been loaded with sodium and depleted of potassium by storage for approximately 48 h at 3 °C, were used. Previous work has shown that 2, 4-dinitrofluorobenzene (DNFB) is a metabolic inhibitor[11], raises intracellular ADP levels[9,12], and induces sodium pump-mediated Na:Na exchange in this preparation[9]. The experiments that follow show that DNFB does not appreciably alter Na:K exchange.

Strophanthidin-sensitive K influx was determined in muscles treated with DNFB to raise their ADP levels, and in controls. Figure 1 shows that elevation of ADP levels had only a small effect on K influx (there was little effect on either $V_{\max}$ or K_m). If Na:Na exchange, which we know to arise in such muscles (see ref. 9 and Fig. 2), actually took the place of Na:K exchange, then strophanthidin sensitive K influx should have been inhibited by induction of Na:Na exchange. There is no indication that this happened.

The sodium pump operates in markedly electrogenic fashion in sodium-loaded frog sartorius muscle fibres[14]. Thus, strophanthidin-sensitive membrane current can be used as another index of the extent of Na:K exchange, assuming that Na:Na exchange is electroneutral[1, 5, 9]. Table 1 lists measurements of membrane potential in a second series of sodium loaded muscles, bathed in 10 mM K low-chloride Ringer's. DNFB did not significantly alter ouabain-sensitive membrane potential or membrane resistance. We conclude, therefore, that elevation of ADP did not alter the current generated by the sodium pump, further evidence against enhancement of Na:Na exchange at the expense of Na:K exchange.

Table 1 Effect of DNFB on the ouabain-sensitive potential, and membrane resistance, of frog sartorius muscles

	Membrane potential (mV)	
	Control	DNFB-treated
Without ouabain	−67.7±1.6 (5)	−72.5±2.0 (5)
With ouabain	−49.3±2.1 (5)	−56.8±2.2 (5)
Ouabain-sensitive	18.4±2.6	15.7±2.9
	Membrane resistance (kΩ cm^2)	
	Control	DNFB-treated
Without ouabain	4.9±0.5 (4)	3.8±0.4 (4)
With ouabain	4.8±0.6 (4)	4.3±0.6 (4)

Values are means ± s.e.m. with the number of muscles indicated in parentheses. The difference in ouabain-sensitive potential between control and DNFB-treated muscles was not significant ($P > 0.2$), nor was the effect of DNFB and/or ouabain on membrane resistance ($P > 0.1$). Muscles were loaded with sodium by storage for 48 h at 3 °C in K-free Ringer's solution. Apart from this loading solution, all other solutions were low in chloride (10 mM) with isethionate used as a substitute. Low-chloride solutions were used to increase membrane resistance and to prevent the ouabain-induced membrane conductance increase described by Geduldig[15]. Washout of internal Cl from the muscle was by 3 h storage at 3 °C in K-free, low-Cl Ringer's Muscles were next equilibrated with K by storage, still at 3 °C, in 10 mM K, low-Cl Ringer's for 45 min. Muscles from 18 frogs were then exposed to 10 mM K, low-Cl Ringer's at room temperature, and muscles from another 18 frogs to a similar solution containing 0.38 mM DNFB. Of each muscle pair from the same frog, one member was exposed to 10^{-4} M ouabain as well. Membrane potential measurements were begun after 20 min and continued for another 20 min. (Since the DNFB effect peaks after 30 min exposure[9], this procedure bracketed the period of maximum Na:Na exchange.) Conventional 3M KCl-filled microelectrodes of approximately 3 mV tip potential and 20 MΩ resistance were used. Two microelectrodes we re-used to determine membrane resistance—one to pass current and the other to record steady-state membrane potential deflection. Input resistance and space constants were obtained from a semi-log plot of potential deflection against electrode separation. Membrane resistance could then be calculated by one-dimensional cable analysis, assuming internal resistance to be 169 Ω cm (ref. 16).

The above argument rests on a comparison made between separate batches of muscles, those used for K influx measurements compared with those used for Na influx measurements. A direct quantitative comparison of the two exchange mechanisms is therefore of value. Strophanthidin sensitive ^{22}Na influx (an index of pump-mediated Na:Na exchange) and strophanthidin-sensitive ^{42}K influx (an index of Na:K exchange) were measured in the same muscles. Figure 2 shows values for Na and K influxes in DNFB-treated and untreated muscles, with and without strophanthidin present. Strophanthidin-sensitive differences are plotted on the right. There is marked stimulation of Na influx (Na:Na exchange) but only slight inhibition of K influx (Na:K exchange). Assuming the pump's exchange rate to be 3:2 per turnover for normal Na:K exchange, and 3:3 when the pump is engaged in Na:Na exchange, then the observed K influx decrease of 1.6 μmol g^{-1} h^{-1} should result in the induction of just under 2.4 μmol g^{-1} h^{-1} Na influx by direct substitution. Since Na influx increased by 8.5 μmol g^{-1} h^{-1} it seems that a substantial amount of 'new' Na:Na exchange must have occurred in addition to ongoing Na:K exchange unless there is a vast difference in turnover rate between the two modes. The work of Garrahan and Glynn in red blood cells[1,2], as well as our own in frog muscle (unpublished) suggests that the turnover rate in the two modes is, in fact, comparable. (In addition, we have evidence that DNFB causes a 20% inhibition of the (Na+K)ATPase isolated from frog muscles, suggesting that some reduction of K influx could result from DNFB treatment, and that the actual discrepancy between Na influx 'gained' and K influx 'lost' may be even larger than observed here.) In any case, Na:K exchange is not much affected by elevation of ADP levels, despite marked stimulation of pump-mediated Na:Na exchange.

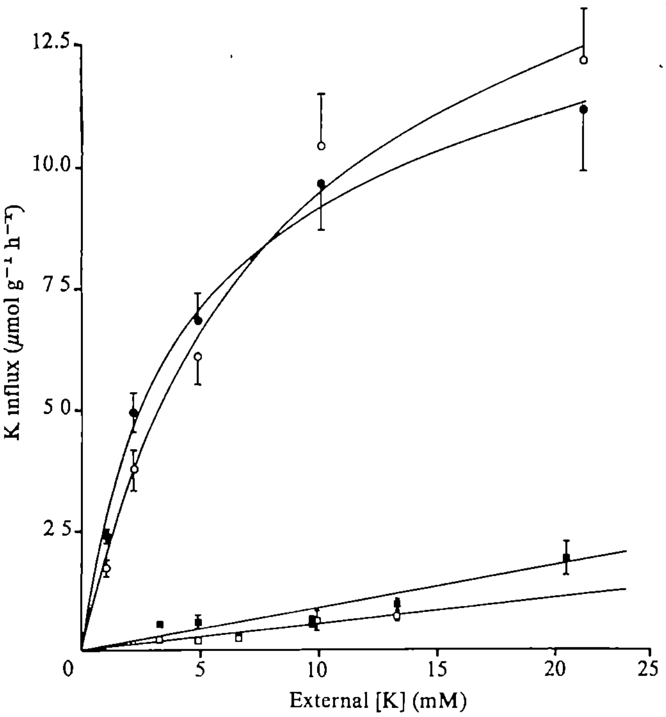

Fig. 1 Unidirectional K influx as a function of external [K] in DNBF-treated and untreated muscles. Values are means±s.e.m. □, ■, muscles exposed to 10^{-4} M strophanthidin; ○, ●, muscles not exposed to strophanthidin. Open symbols refer to muscles treated for 30 min with 0.38 mM DNFB; closed symbols refer to untreated muscles. The data obtained in the presence of strophanthidin were fitted by straight lines; the strophanthidin sensitive fluxes were fitted by rectangular hyperbolae. Muscles were stored in K-free Ringer's solution in the cold for approximately 48 h before the experiment. All solutions contained 5 mM Ba (hence the low level of strophanthidin-insensitive K influx)[13], and 10^{-7} g ml^{-1} tetrodotoxin to prevent twitching. K was elevated by substitution for Na, which was 115 mM in K-free Ringer's solution. Muscles were exposed to ^{42}K for 25 min (K influx is essentially linear for at least 1 h)[7] and total intracellular radioactivity was determined by back extrapolation to zero time of the linear portion of the washout curve. Parameters were: maximal influx, 10.92 ± 1.03 μmol g^{-1} h^{-1}, and K_m 3.19 ±0 73 mM, for control muscles; and maximal influx, 14.94 ±1.70 μmol g^{-1} h^{-1}, and K_m, 6.76±1.49 mM, for DNFB-treated muscles. There was a marginally significant effect of DNBF on both parameters ($0.05 > P > 0.02$).

To summarise, our experiments were designed to determine whether Na:Na exchange occurs in addition to, or in substitution for, ongoing Na:K exchange. We examined whether ouabain sensitive membrane potential and strophanthidin-sensitive K influx (both indices of pump-mediated Na:K exchange) could be inhibited by induction of Na:Na exchange, and found little evidence of inhibition. Our conclusion is that Na:K exchange can continue unaltered when Na:Na exchange is induced by the presence of ADP. This effect of intracellular ADP on the mode of operation of the sodium pump is in marked contrast to that of external potassium. Garrahan and Glynn[2] have shown in red blood cells that reduction of $[K]_o$ stimulates Na:Na exchange at the expense of Na:K exchange, and we have similarly found that raising $[K]_o$ to 10 mM reduces pump-mediated sodium influx into DNFB-treated skeletal muscle to low levels[17]. Further strong evidence in favour of interconvertibility of Na:K and Na:Na exchange is provided by the work of Bodemann and Hoffman[18].

The following qualitative kinetic picture of the sodium pump can now be drawn. In muscle cells with normal (that is, low) levels of ADP, bathed in K-free Ringer's solution, sodium

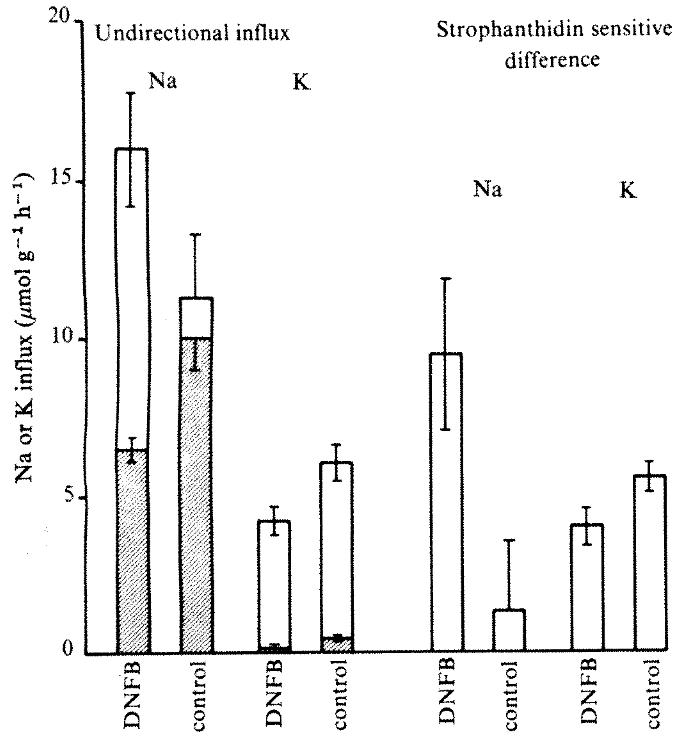

Fig. 2 Simultaneous determination of Na and K influxes. Values are means ± s.e.m. Protocol as for Fig. 1 except that both ^{22}Na and ^{42}K were present in the labelling solution, and that [K] was kept at 2.5 mM. At this [K], ouabain sensitive Na influx is reduced to about two thirds of its maximum value observed in K-free Ringer's. Hatched areas represent fluxes in the presence of 10^{-4} M strophanthidin. Washout samples were counted to determine total counts and then recounted after decay of the ^{42}K (more than 11 half lives). We found no long-lived contaminant in the ^{42}K used. Na influx values were not corrected for efflux during the uptake period, since the rate coefficient for efflux in DNFB is not constant[9]. Efflux is smaller in untreated muscles than in those treated with DNFB, however. Thus the strophanthidin-sensitive Na influx in DNFB will be underestimated (using the peak rate constant for efflux in DNFB, this underestimation was calculated to be about 20%) to a greater extent than that in untreated muscles. Consequently the stimulation of Na:Na exchange should, if anything, be even more pronounced than the uncorrected data suggest.

pump molecules are mostly inactive. As the external potassium concentration is raised, pump activity will increase toward saturation, thus reducing the silent fraction. At a given $[K]_o$, the silent fraction can be made to engage in Na:Na exchange to an extent determined by intracellular [ADP], while the previously active fraction continues to engage in Na:K exchange. Further elevation of $[K]_o$ in the presence of high intracellular ADP levels continues to recruit an increasing fraction of the pump population into Na:K exchange, but now at the expense of both the silent (if any) and the Na:Na exchanging fraction. Thus, induction of Na:Na exchange by ADP is additive with respect to ongoing Na:K exchange, as shown here, but induction of Na:Na exchange by removal of external potassium[2] is substitutive with respect to ongoing Na:K exchange.

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Independent mechanisms of cyclic AMP and glucocorticoid action

IN eukaryotic cells, cyclic adenosine monophosphate (cyclic AMP) acts as the intracellular mediator for several hormones (for example, adrenaline and certain polypeptide hormones)[1] and plays an important part in the modulation of cell proliferation[2,3]. This cyclic nucleotide is known to function by activating a protein kinase[4,5] which mediates the phosphorylation of specific proteins, thereby modifying their biological activity. The activation of protein kinase may, in fact, be the sole mechanism of action of cyclic AMP in mammalian cells[6,7]. Glucocorticoids, and steroid hormones in general, act through a mechanism[8,9] which involves the binding of the hormone to specific cytoplasmic receptor proteins, the translocation of the receptor–steroid complex into the cell nucleus and the association with chromatin. The cellular responses to glucocorticoids and cyclic AMP are quite similar in several systems and the potentiating effect of the steroid on cyclic AMP-mediated processes is often referred to as 'permissive effect' (for reviews see refs 10 and 11). In several cell types glucocorticoids have been reported to increase, although moderately, the intracellular concentration of cyclic AMP (refs 12–15). These observations suggest that the cyclic nucleotide and glucocorticoids could have some distal step(s) in common in their mechanism of action. We have tested this hypothesis by cloning responsive cells in the presence of both effectors and report here that resistance to cyclic AMP and glucocorticoids occurs independently.

Our experiments were carried out with cultures of S49.1 mouse lymphoma cells in which both glucocorticoids and cyclic AMP (or its dibutyryl derivative, db cyclic AMP) inhibit cell proliferation and, on prolonged exposure, cause the cells to die[16–19]. Mutant cells resistant to growth-inhibitory and cytolytic effects have been isolated and most of them have defects related to known steps in the action mechanism of the two effector types[7,20–23]. One would therefore expect that most of the mutants of each type

Fig. 1 Dexamethasone binding in cytosol preparations of S49.1 clones. The clones (experiment of Table 2) grown in the presence of db cyclic AMP and dexamethasone (△, ▽) as well as representative clones from the other groups of plates (no selection, ●; selected in db cyclic AMP, ○; selected in dexamethasone, □) were grown up in suspension cultures. Cytosols were prepared and incubated with various concentrations of ^{3}H-dexamethasone (26 Ci mmol^{-1}, Amersham)[27]. The specifically bound steroid is plotted as a function of free steroid.

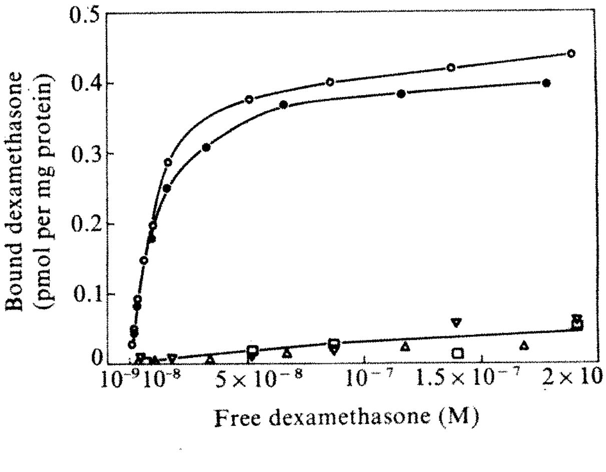

Table 1 Sensitivity of S49.1 clones isolated by single-step selection

Cloning in	Number of clones tested	Number of clones sensitive to Dexamethasone	db cyclic AMP
Dexamethasone	8	0	8
db cyclic AMP	20	20	0

S49.1 cells[14] were grown in Dulbecco's modified Eagles' medium supplemented with 10% foetal calf or horse serum and cloned over a feeder layer of mouse embryo fibroblasts[24,25] in soft agar containing 10^{-7} M dexamethasone or 0.5 mM db cyclic AMP and 0.2 mM theophylline. Clones were grown up and tested for sensitivity in suspension culture by seeding cells at a density of 3×10^5 ml^{-1} in medium containing either 10^{-7} M dexamethasone or 0.5 mM db cyclic AMP and 0.2 mM theophylline. After 3 d of incubation, cell viability was determined with Trypan blue at a concentration of 0.2%. Clones were designated sensitive if > 90% of the cells stained blue. At least 90% of resistant cells or cells incubated without drug excluded the dye.

should not show cross resistance. This is seen in Table 1 for several S49.1 clones which have been isolated by independent procedures. Since most glucocorticoid-resistant mutants carry defects in the steroid receptor[22,23], the possibility that the steroid receptor is involved directly in the response to the cyclic nucleotide is excluded. There could exist at a relatively low frequency, however, a subclass of mutants which differ from those described thus far and are defective in some mechanism common to both glucocorticoids and cyclic AMP. Such cells should be doubly resistant and should be selected preferentially if both agents were used together.

Table 2 shows a cloning experiment in which both the steroid and db cyclic AMP were used. Here the cloning efficiency was extremely low compared with cloning in the presence of either one of the agents; only two colonies were seen. Significantly, this incidence is nearly the product of the frequencies at which singly resistant clones occurred.

The most plausible explanation for this observation is that the 'doubly resistant' cells contain two independent mutations. These cell clones were therefore examined for the phenotypes of resistance. The cells of both clones were identified as deficient in glucocorticoid-specific receptors (Fig. 1). In extracts of these cells however, protein phosphokinase activity was stimulated by cyclic AMP in a manner indistinguishable from the wild type (data not shown). Growth experiments in suspension cultures revealed that the two clones obtained in the double selection experiment of Table 2 were still growth inhibited by the cyclic nucleotide (Fig. 2). The half-maximum effect was between 0.05 and 0.1 mM db cyclic AMP (data not shown). Consistent with this growth inhibition, these clones appeared as very small colonies in the experiment of Table 2 and would probably not have been picked for further propagation in a single selection experiment in which a fair number of more highly resistant mutants with defects in protein kinase are routinely recovered[25]. The preferential accumulation of cells in the G_1 phase of the cell cycle was observed by flow cytometry[28] on exposure of the mutant cells to the cyclic nucleotide (data not shown). While 0.3 mM db cyclic AMP completely arrested wild-type cells in G_1 after 24 h (ref. 17), the effect on the mutant cells was less pronounced. This is consistent with the intermediate growth inhibition (Fig. 2). These growth characteristics and the presence of cyclic AMP-responsive phosphokinase show phenotypic similarities between these mutant clones and the recently isolated cyclic AMP unresponsive 'deathless' cells[29]. These 'deathless' mutants are growth inhibited, but not lysed, by cyclic AMP, however, they are fully sensitive to steroid-induced cytolysis.

Table 2 Incidence of resistance to dexamethasone and db cyclic AMP

Agent present during cloning		Cells applied per plate	Colonies grown per plate	Mean frequency of resistant cells*
Dexamethasone	db cyclic AMP			
–	–	10^2	45, 50, 53	
+	–	10^4	22, 24, 27	5.0×10^{-3}
–	+	10^6	45, 49, 52	9.9×10^{-5}
+	+	10^6	1,1,0,0,0, 0,0,0,0,0	4.1×10^{-7}

*Corrected for plating efficiency of 49%.

Recently cloned S49.1 cells were treated for 24 h with 0.75 µg ml^{-1} ICR 191 (a gift from Dr H. R. Creech). Surviving cells were allowed to grow up in the absence of drugs and were used for the cloning experiment (compare with Table 1). Plates with additions contained dexamethasone at 10^{-7} M and 0.5 mM db cyclic AMP plus 0 2 mM theophylline. The expected frequency of doubly resistant mutants is $5.0\times10^{-3}\times9.9\times10^{-5}=5.0\times10^{-7}$ if each marker is acquired in a distinct and independent step[26].

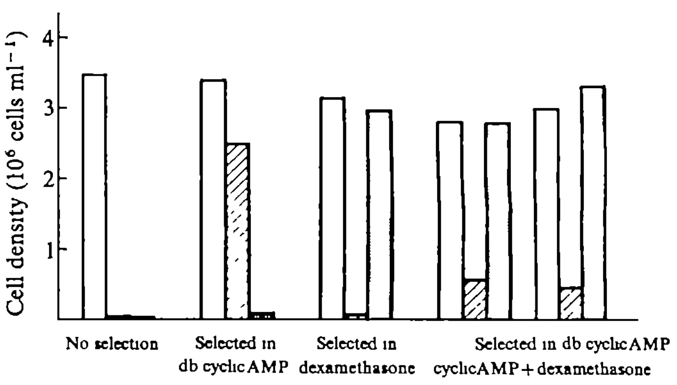

Fig. 2 Growth of S49.1 clones in the presence of db cyclic AMP and dexamethasone. Clones from the experiment of Table 2 (the same as used for Fig. 1) were grown in suspension culture and seeded at a cell density of 2×10^5 ml^{-1} in medium containing either 10^{-6} M dexamethasone (stippled bars) or 0.5 mM db cyclic AMP and the phosphodiesterase inhibitor RO 20-1724 at a concentration of 30 µM (hatched bars). The density of viable cells was determined after 72 h. Control cultures are represented by open bars.

The experiments described here indicate two independent lesions for steroid resistance and decreased cyclic AMP sensitivity, respectively, in the 'doubly resistant' mutants. This implies that there is no common mutable biochemical step in the two processes from effector–cell interaction to cytolysis in the cell system studied here. We cannot exclude, however, the possibility that mutations in a biochemical process common to cyclic AMP and steroid-mediated cytolysis occur at a frequency too low to be detected by our technique. Alternatively, such a common biochemical process could be required for cell viability. If so, mutations affecting that putative process would result in cell death and mutants could therefore not be obtained, except as conditionally lethal mutants. Nevertheless, there is synergism between cyclic AMP and glucocorticoids in S49.1 lymphoma cells. If these cells are pre-exposed to db cyclic AMP and thereby arrested in the G_1 phase of the cell cycle they are much more susceptible to the cytolytic effect of the steroid than untreated cells[18]. There must therefore exist, at some level, interaction between both effector systems in these cells as well as possibly in other mammalian cells.

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The shape of the ultraviolet inactivation curve for transforming DNA

THE very unusual inverse square kinetics of ultraviolet inactivation of transforming DNA were first reported by Rupert and Goodgal[1], and subsequently there was speculation that these kinetics result from blocks to recombination formed by ultraviolet lesions in the DNA[3] (ref. 2). We show here that when the recipient cell lacks both excision and postreplication repair, the ultraviolet inactivation of transforming DNA becomes a simple exponential rather than an inverse square relation. The data suggest that the square root kinetics observed with repair-proficient recipient cells result from repair processes themselves rather than from the recombination between irradiated transforming DNA and recipient DNA.

The inverse square relation, shown for transforming DNA from *Haemophilus influenzae*[1] and *Bacillus subtilis*[2], is: $\sqrt{(N_0/N)}=1+CD$, where N_0 and N are the number of transformants from unirradiated and irradiated DNA, respectively, D is the dose and C is a constant which depends on the genetic marker, the particular DNA preparation, the wavelength of the radiation and the repair capacities of the host[1,3,4]. The equation fits the data except for very large doses, where the inactivation becomes close to exponential[5]. Two theoretical formulations for the inactivation of transforming DNA have been based on the idea that recombination of a piece of DNA can take place up to but not including an ultraviolet lesion[2,6] After the first increment of dose, the 'targets' for further inactivation are the sections of DNA molecules between the genetic marker and the nearest lesion. Further inactivation takes place only when a lesion occurs in one of these sections. Thus with increasing dose the target decreases in size, and a larger and larger increment of dose is necessary for further inactivation. The theoretical formulations, although slightly different, both approximate to the empirically obtained equation[1], but are based on an incorrect assumption, namely that pyrimidine dimers in transforming DNA, responsible for almost all the inactivation by ultraviolet radiation[5,7], are not integrated into the recipient genome. It was first shown that dimers are integrated into *H. influenzae* recipient DNA by a demonstration that irradiated transforming DNA which was integrated by the criterion of marker linkage could be enzymatically photoreactivated[8]. It was later found that integrated DNA containing dimers can kill the recipient cell[9], and that excision of dimers takes place only after the damaged DNA has been integrated[10–13].

Excision repair might be considered to be responsible for the square root relationship, with the following rationale. Because transforming DNA is integrated into the recipient genome as a single strand[14], there is a finite probability that excision repair not only may eliminate damage, but may also eliminate the genetic marker assayed in transformation. Following a given increment of dose, whether the repair process eliminates the marker or helps the marker to survive depends on the distance between the marker and the nearest ultraviolet lesion (usually a pyrimidine dimer), and also on the length of the region excised, assumed to be variable from one repaired region to another. After a second increment of dose, the only transforming DNA molecules with a still lower probability for transformation are those with an ultraviolet lesion between the marker and the previous lesion. But, excision repair alone cannot provide the explanation for the shape of the inactivation curve for transforming DNA, since the square root relationship also holds for recipients that are lacking in excision ability[4,15,16].

It has been suggested that postreplication repair might be the cause of some loss of donor genetic information, and thus be responsible for the shape of the inactivation

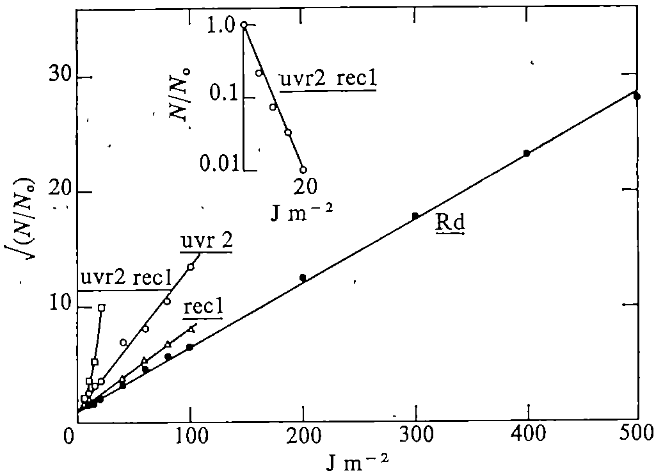

Fig. 1 Inactivation of transforming DNA assayed on four different streptomycin-sensitive *H. influenzae* strains, *Rd* (wild type), *recl-1* (ref. 20), *uvr2* and *uvr2recl* (ref. 21). N and N_0 are the number of transformants obtained from irradiated and unirradiated DNA. Transforming DNA from cells resistant to 250 μg ml^{-1} streptomycin was purified as previously described[22] and irradiated at 10 μg ml^{-1} in 0.15 M NaCl plus 0.015 M trisodium citrate. Irradiation at 254 nm was performed as before[4]. Cells were made competent by a modification of Stuy's method[23]. They were grown with aeration in supplemented brain heart infusion medium[4] to an optical density of 0.44 at 675 nm, and then left standing at 37 °C for 90 min without aeration They were then diluted a factor of eleven into 10 mM phosphate buffer, *p*H 7.0, plus 0.15 M NaCl, and shaken at 30 °C for 60 min. They were next exposed to 1 μg ml^{-1} transforming DNA at 37 °C for 30 min. Growth medium (2 to 5 ml) was then added to each 1 ml of cells, and the cells were grown for 2–3 h before being plated in streptomycin-containing agar medium. The larger volume was used for the *recl* strains, in order to obtain statistically significant numbers of transformants, and ten platings of 1 ml each were made for each point. Each of these assays (all obtained with the same set of irradiated DNAs) were repeated at least three times and the data averaged. The minimum number of *recl* or *uvr2recl* colonies counted per point was 70. The approximate frequencies of transformation by unirradiated DNA in these experiments were 0.7×10^{-2} (*Rd*), 0.5×10^{-2} (*uvr2*), 0.5×10^{-7} (*recl-1*) and 0.2×10^{-7} (*uvr2recl*).

curve[14]. Postreplication repair in *H. influenzae* has been shown to involve extensive exchange between new and old strands[17], during which the transforming DNA marker integrated into only one strand could be eliminated with a probability dependent on the distance between the marker and the nearest lesion, and on the (variable) length of the exchange. A test of the hypothesis is to measure the survival of irradiated transforming DNA on a mutant strain such as *recl* (ref. 18) deficient in postreplication repair[17,18]. Earlier attempts at this experiment were defeated by the extremely poor transformation of the mutant (around 10^{-6} the frequency of the wild type). Recently it has been found that with a different competence regime, the transformability of the *recl* strains, but not other strains, is markedly improved[20], making the measurement feasible.

Figure 1 shows data plotted in the square root plot for ultraviolet inactivation of transforming DNA assayed on four different *H. influenzae* recipients. The data on three of the recipients, the wild-type (strain *Rd*), postreplication repair-deficient *recl* and excision-deficient *uvr2*, fit the straight line relationship, whereas on the double mutant *uvr2recl* recipient the data deviate from the straight line. Moreover, the transforming DNA assayed on *uvr2recl* seems to be inactivated exponentially, as seen in the insert of Fig. 1.

I interpret the data to mean that excision and/or postreplication repair in the recipient is responsible for the inverse square relationship. When repair is absent the inactivation becomes exponential, as though there is a critical region of DNA surrounding the marker which must be free of damage for transformation to be successful.

Excision repair increases survival of transforming DNA more than does postreplication repair, as seen in the fact that the DNA assayed on *recl* seems only a little more sensitive than on the wild-type strain *Rd*, but on *uvr2* the DNA seems considerably more sensitive. Postreplication repair in *H. influenzae* is not an inefficient process, as judged by its effect on survival following ultraviolet irradiation of cells[21]. Thus postreplication repair may eliminate the transforming DNA marker almost as often as it increases its survival.

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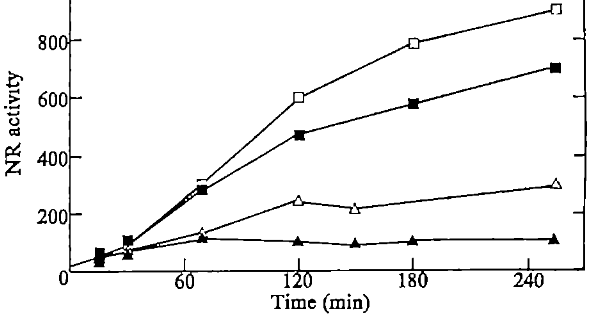
Psoralen and near ultraviolet light; a probe for study of control of protein synthesis

THE study of the control of protein synthesis in eukariotic cells usually involves the use of chemical inhibitors of macromolecule synthesis[1]. The drawback of these procedures is that the inhibitors have side effects which do not allow conclusive interpretations. Furthermore, the precise timing of the inhibitor effect is not always possible due to the lack of knowledge of the effective *in vivo* concentration and the rate of its entry into the cells. Furocoumarins (psoralens) photosensitise biological systems to near ultraviolet (NUV) light (300–400 nm) (refs 2–4). Psoralen and its derivatives react with pyrimidine bases in DNA when irradiated with NUV light to form monoadducts and DNA crosslinks[5,6], the latter being the main lesion responsible for the biological damage in bacteria and mammalian cells[4,7]. Since the photoreactions in the cells are specifically with DNA[8,9] and lead to its immediate inactivation as a template for DNA and RNA synthesis[9], we have considered the use of this system for the study of control of protein synthesis. The degree of inactivation of DNA can be easily controlled by varying the NUV light dose, thus avoiding the complications involved in the use of drugs.

The well documented[10] development of nitrate reductase (NR) activity in the XD cell line of tobacco, in response to nitrate, was chosen as the system under study. The cells were grown as previously described[11] on chemically defined, nitrate-less M-ID medium supplemented with 5 mM L-glutamine (Gln cells) as a sole nitrogen source. Cells were grown to mid-logarithmic phase, collected by filtration and resuspended in fresh nitrate-less M-ID medium at 1 g per 10 ml. 4,5′8-trimethylpsoralen (Paul B. Elder Co.) was added to a final concentration of 5×10^{-6} M. The cells were incubated in the presence of psoralen for 5 min, and then irradiated for the indicated duration with two tubular fluorescent black light lamps (UV Products, lamp 50058) held in a reflector. Maximum emission of the lamps was at about 360 nm and the incident flux was 20 J m^{-2} s^{-1} as measured by a calibrated black light meter (Model J-221, UV Products). During irradiation, the cell suspensions were continuously stirred. At the end of the irradiation period, samples of the cell suspensions were removed and were either diluted into nitrate-less M-ID medium supplemented with 10 mM KNO_3, or kept undiluted and given KNO_3 to a final concentration of 10 mM, when induced, or non-induced cells were used, respectively. Cells not treated were used as control. Following irradiation, the cells were shaken for the duration of the experiment. The NR activity *in situ* was determined at time intervals by the procedure described by Ferrari *et al*[12]. Enzyme activity is expressed as nmol NO_2^- formed per h per g fresh weight.

The effect of psoralen + NUV light on the induction of NR activity induced by nitrate in Gln cells is shown in Fig. 1. Increasing the radiation dose reduced the rate of develop-

Fig. 1 The inhibitory effect of psoralen plus NUV light on the induction of NR activity. Cells were grown in nitrate-less M-ID medium supplemented with 5 mM L-glutamine. The cells were collected and resuspended in nitrate-less M-ID. KNO_3 (10 mM) was added to part of the cell suspension and used as control (□). The rest of the cell suspension was exposed to psoralen (final concentration 5×10^{-6} M) and irradiated for 1 min (■); 3 min (△), and 5 min (▲). KNO_3 was added to the treated cells (final concentration 10 mM) at the end of the irradiation period. The NR activity *in situ* was determined at the indicated times.



ment of NR activity and markedly lowered the steady state level of the enzyme. The two effects could be observed as early as 15–30 min after exposure of the cells to nitrate. There was no effect of either psoralen alone or irradiation alone (data not shown). Only the combination of psoralen and NUV light at the proper dose was inhibitory to the development of enzyme activity.

The effect of psoralen plus NUV light on partially induced cells is shown in Fig. 2. Irradiation for 1 min strongly inhibited further development of the enzyme as compared with control cells. Higher radiation doses caused the initial level of activity to decline in time.

Based on the target specificity of psoralen plus NUV light[8] we interpreted the results shown in Figs 1 and 2 as follows. Exposure of cells to psoralen plus NUV light just before nitrate addition (non-induced cells) or during the induction process (induced cells) inhibits the synthesis of functional mRNA, thereby inhibiting the development of NR activity. We further suggest that in cells growing on L-glutamine as a nitrogen source there is none or very little mRNA coding for the NR protein complex or for an essential subunit of the enzyme. If mRNA coding for the enzyme were present in Gln cells one would expect an initial development of NR activity upon exposure of the cell to nitrate, which is insensitive to the effect of psoralen plus NUV light. The inhibitory effect of psoralen plus NUV light would be observed gradually as old mRNA decays and the synthesis of new mRNA is inhibited. Such a situation is summarised in Fig. 3. It was previously shown[13] that tungsten (W), an analog of molybdenum (Mo), inhibits the development of NR activity in XD cells due to the formation of non-functional enzyme. The non-functional enzyme can be made functional to a certain extent *in vivo* in the absence of protein synthesis (presence of cycloheximide) by adding excess Mo. Thus, it was assumed that the mRNA present in W-treated cells and translated to a non-functional enzyme, would be translated to a functional enzyme following exposure to psoralen plus NUV light and addition of Mo. It can be seen from Fig. 3 that this was indeed the case. The low level of NR activity in cells which were treated with W increased following exposure to psoralen + NUV light and addition of excess Mo. The increase cannot be accounted for by the activation of pre-existing non-functional enzyme, as can be judged by cells treated with cycloheximide before the addition of Mo.

We conclude that exposure to psoralen plus NUV light inhibits the synthesis of mRNA coding for NR or an essential subunit of the enzyme, thereby inhibiting the synthesis of the enzyme. The translation of pre-existing mRNA is not affected. We suggest that the combination of psoralen and NUV light can be used to distinguish between transcriptional and post-transcriptional controls of protein synthesis.

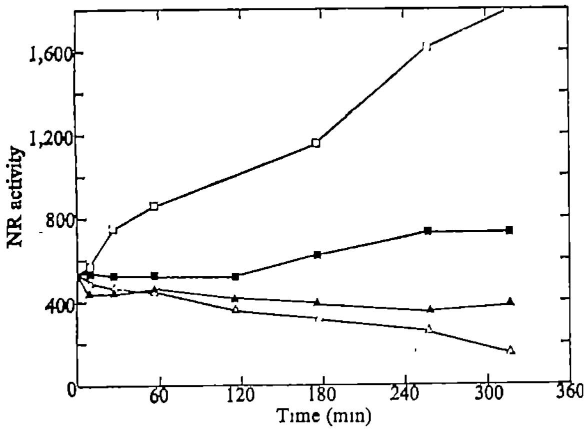


Fig. 2 The effect of psoralen plus NUV light on NR activity of partially induced cells. Cells were allowed to develop NR activity for 24 h and were then collected and treated as described in Fig. 1. Control (□), 1 min irradiation (■), 3 min irradiation (▲), 5 min irradiation (△).

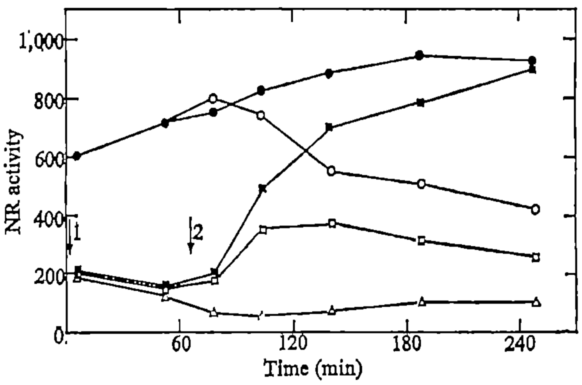


Fig. 3 Translation of pre-existing mRNA following exposure to psoralen plus NUV light. Cells were allowed to develop NR activity in the absence (●, ○) and presence (■, □, △) of 2×10^{-4} M sodium tungstate. At zero time, arrow no. 1, cycloheximide was added to one of the cell suspensions (△) to a final concentration of 10 μg ml^{-1}. Arrow no. 2 indicates: first, exposure of the cells to psoralen plus 5 min irradiation (○, □), and second, the addition of sodium molybdate to a final concentration of 2×10^{-4} M to all of the cell suspensions (●, ○, ■, □, △). At the indicated times NR activity *in situ*, was determined.

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Genetic recombination through protoplast fusion in *Streptomyces*

MEMBERS of the genus *Streptomyces* yield over 60% of known antibiotics[1], including more than 70 commercial products[2], as well as at least one very important enzyme (glucose isomerase). Members of the related genera *Nocardia* and *Micromonospora* produce some further valuable antibiotics (rifamycins, gentamicins). Recombination through conjugation is widespread in these actinomycetes[3] but only to a very limited extent has it been applied to strain improvement. The main reason for its neglect has probably been the necessity to introduce selectable (for example drug resistance) or more generally counter-selectable (auxotrophic) markers into the strains to be crossed in order to identify recombinants occurring at frequencies of 10^{-6} or lower among predominantly asexual progeny. The marking of strains is time-consuming; moreover it may induce deleterious mutations along with the markers, and these will usually go undetected since auxotrophic mutations themselves often depress antibiotic yield. In certain cases, recombination occurs so rarely that single markers in each parent cannot be used to distinguish it from reverse mutation[4], while in some strains

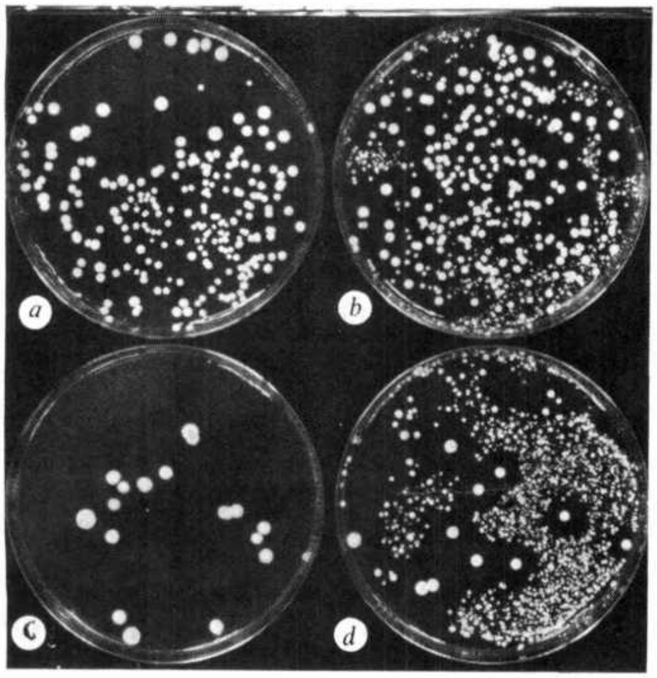

Fig. 1 Platings of *S. acrimycini* protoplasts on regeneration medium after dilution in water (*a*, *c*) or medium P (*b*, *d*) at dilutions 10^{-1} (*a*, *b*) and 10^{-2} (*c*, *d*). Note the large colonies derived from osmotically stable non-protoplasted units, occurring in equal numbers on both series of plates, and the much more numerous colonies arising from regenerated protoplasts after dilution in medium P. The latter are severely inhibited, especially at lower dilution, by the precociously developing non-protoplast colonies.

no recombination has been detected[3]. The availability of a simple generally applicable procedure to recombine actinomycete strains at high frequency would therefore greatly facilitate the routine use of recombination in strain improvement. We report here such a procedure for *Streptomyces* which depends on polyethylene glycol (PEG)-induced protoplast fusion and regeneration. Recombination frequencies achieved by this technique are so high that selectable markers could be dispensed with, at least in certain kinds of strain improvement programmes.

Protoplasts of five species were prepared and regenerated by procedures slightly modified from those of Okanishi, Suzuki and Umezawa[5]. 25 ml samples of medium S containing 1–4% glycine, depending on the strain, were inoculated with 10^7–10^8 spores and incubated with shaking in 250 ml Erlenmeyer flasks at 30 °C for 3–4 d. Growth was progressively retarded, and the efficiency of conversion of mycelium to protoplasts increased, with increasing glycine concentration. For routine protoplast preparation, mycelium was therefore collected from flasks with a glycine concentration which markedly inhibited growth but which allowed a reasonable yield of mycelium. After two washes at room temperature with 0.3 M sucrose, mycelial pellets (usually consisting of spherical aggregates about 1 mm in diameter) were re-suspended in 1.5 ml of lytic enzyme mixture and incubated at 32 °C until clouds of protoplasts were liberated from the mycelial mass on gentle swirling of the liquid (60–90 min). 4 ml of medium P was added; the suspension was pipetted vigorously several times to aid release of protoplasts from the still largely coherent mycelial mass, and filtered through cotton wool (sometimes followed by Nucleopore filtration). The protoplasts were pelletted by centrifugation at 1,000*g* for 5–10 min, resuspended in 4 ml of medium P and again pelletted and resuspended.

For studies of protoplasting and regeneration efficiency, samples of the suspensions were diluted in parallel in medium P (which contains 0.3 M sucrose) or in water and plated on R2 regeneration medium supplemented with required growth factors. (In preliminary work, the R1 and R2 media[5] were assessed; somewhat higher regeneration frequencies were obtained with R2 and this was therefore adopted.) Colonies from water-diluted suspensions arose from osmotically stable and presumably non-protoplasted units (mycelial fragments produced by sonic disruption of control cultures equilibrated in medium P were 100% osmotically stable), while the excess of colonies from comparable dilutions in medium P represented regenerated protoplasts. For some strains, non-protoplasted units developed precociously so that colony counts after dilution in water or medium P were at first the same; later, numerous protoplast colonies appeared on the plates that received osmotically protected suspensions. In most strains the protoplast colonies soon reached the size of the non-protoplast colonies. In *S. acrimycini*, however, non-protoplast colonies inhibited development of the protoplast colonies, which were largely suppressed on high-density plates (Fig. 1). Non-protoplasted units typically represented 0.1–1% of total colony-forming units when the parent culture was grown with an optimal glycine concentration. With lower concentrations, the proportion was higher; it was essentially unchanged by filtration through 2.1-μm Nucleopore filters after the routine cotton-wool filtration and so this step was usually omitted. Protoplasts varied so greatly in size, many possibly being too small to contain a genome, that little confidence was placed in microscopic counts; rough estimates of regeneration frequency were lower than the maximum of 40–50% reported by Okanishi *et al.*[5].

Protoplast fusion induced with PEG has been described in bacilli[6,7] but we closely modelled our procedure on that of Pontecorvo, Riddle and Hales[8] for animal cells. Equal volumes (0.5–2 ml: half to a fifth of the yield of a flask) of protoplast suspensions of the two strains were mixed and, after centrifugation, the pellet was thoroughly drained and rapidly suspended in 0.5 ml of PEG 1540 1w+1.4 volumes medium P containing 15% dimethylsulphoxide (DMSO). After 1 min, 0.5 ml of PEG 1w+3 volumes of medium P was added and, after a further 3 min, 4 ml of medium P. Control pellets were suspended in 5 ml of medium P. The suspensions (containing flocculent protoplast aggregates after PEG treatment) were centrifuged, each pellet was resuspended in 0.25 ml of medium P, and the liquid was poured over a single plate of R2 medium supplemented with all growth factors required by both parental strains. Gentle spreading of the suspensions was done with a loop, rather than a glass spreader which might have tended to disrupt protoplast aggregates. After incubation of the plates for 4–6 d, spores were collected from the confluent cultures and assayed for parental and recombinant genotypes by standard procedures[9].

For four species, *S. coelicolor*, *S. lividans*, *S. parvulus* and *S. griseus*, recombinants recovered on a medium selecting markers at two loci represented 1–27% of the frequency of the minority parental class, and 1–6% of the sum of both parentals (Table 1). In *S. coelicolor* A3(2), the same high recombinant frequency occurred in the absence of both known sex factors, SCP1 and SCP2 (ref. 10), as in the presence of one (Table 1) or both, suggesting that the recombination occurring following protoplast fusion is independent of sex factor activity and should therefore occur even in wild types that lack sex factors. In *S. acrimycini*, the recombination frequency was only 3×10^{-4}, but this is readily explicable by the inhibition of protoplast by non-protoplast colonies (see above; Fig. 1); a minority of the latter contributed disproportionately to the total spore progeny on the regeneration plates. There was little or no evidence for significant spontaneous protoplast fusion

Table 1 Recombination in five streptomycetes

Strains*	Treatment	Recombinants per minority parent	per majority parent	per total
S. coelicolor A3(2)	Mating	1.0×10^{-4}	1.8×10^{-6}	1.7×10^{-6}
(SCP1$^-$SCP2$^+$ × SCP1$^-$SCP2$^+$)	Protoplasts (no PEG)	7.6×10^{-6}	7.4×10^{-6}	3.8×10^{-6}
	(PEG)	9.9×10^{-2}	9.1×10^{-2}	4.7×10^{-2}
S. coelicolor A3(2)	Mating	9.4×10^{-7}	2.0×10^{-7}	1.7×10^{-7}
(SCP1$^-$SCP2$^-$ × SCP1$^-$SCP2$^-$)	Protoplasts (no PEG)	3.2×10^{-6}	1.5×10^{-6}	1.0×10^{-6}
	(PEG)	20×10^{-2}	8.9×10^{-2}	6.2×10^{-2}
S. parvulus ATCC 12434	Mating	1.8×10^{-5}	1.0×10^{-5}	6.3×10^{-6}
	Protoplasts (no PEG)	3.0×10^{-6}	2.3×10^{-6}	1.3×10^{-6}
	(PEG)	1.2×10^{-2}	1.1×10^{-2}	0.6×10^{-2}
S. lividans 66	Mating	$< 1\times10^{-4}$	$< 1\times10^{-6}$	$< 1\times10^{-6}$
	Protoplasts (no PEG)	$< 1\times10^{-4}$	$< 4\times10^{-7}$	$< 4\times10^{-7}$
	(PEG)	27×10^{-2}	7.2×10^{-2}	6.0×10^{-2}
S. griseus CUB 94	Mating	1.0×10^{-4}	2.4×10^{-6}	2.3×10^{-6}
	Protoplasts (no PEG)	1.5×10^{-4}	2.1×10^{-5}	1.9×10^{-5}
	(PEG)	9.8×10^{-2}	1.1×10^{-2}	1.0×10^{-2}
S. acrimycini IPV 1610	Mating	9.0×10^{-5}	5.1×10^{-5}	3.2×10^{-5}
	Protoplasts (no PEG)	4.0×10^{-5}	1.7×10^{-5}	1.2×10^{-5}
	(PEG)	5.3×10^{-4}	4.9×10^{-4}	2.6×10^{-4}

Compositions (quantities per litre of final medium) of medium S (for mycelial growth), medium P (for protoplast formation) and R2 (for regeneration) were as follows[5]. Medium S: glucose, 10 g; Difco Bacto peptone, 4 g; Difco yeast extract, 4 g; $MgSO_4.7H_2O$, 0.5 g; KH_2PO_4, 2 g; K_2HPO_4, 4 g. Glycine was added to medium S at 1% for *S. coelicolor* and *S. lividans*, 1.5% for *S. parvulus*, 2.5% for *S. acrimycini* and 3.5% for *S. griseus*. Medium P: sucrose, 103 g; K_2SO_4, 0.25 g; $MgCl_2.6H_2O$, 2.03 g; trace element solution (per litre: $ZnCl_2$, 40 mg; $FeCl_3.6H_2O$, 200 mg; $CuCl_2.2H_2O$, 10 mg; $MnCl_2.4H_2O$, 10 mg; $Na_2B_4O_7.10H_2O$, 10 mg; $(NH_4)_6Mo_7O_{24}.4H_2O$, 10 mg), 2 ml; †KH_2PO_4 (0.5%), 10 ml; †$CaCl_2.2H_2O$ (3.68%), 100 ml; †TES buffer (0.25 M, *p*H7.2), 100 ml. Medium R2: sucrose, 103 g; K_2SO_4, 0.25 g; $MgCl_2.6H_2O$, 10,12 g; trace element solution, 2 ml; glucose, 10 g; Difco casaminoacids, 0.1 g; Difco Bacto agar, 22 g; †KH_2PO_4 (0.5%), 10 ml; †$CaCl_2.2H_2O$ (3.68%), 80 ml; †L-proline (20%) 15 ml; †TES buffer (0.25 M, *p*H 7.2), 100 ml.

*CUB, collection of the University of Bradford; IPV, collection of the Istituto Patologia Vegetale, Milan; for *S. lividans* 66 see ref. 14.

†Added after autoclaving.

since recombination frequencies were similar in mixtures of protoplasts untreated with PEG and in standard crosses ('matings') made on supplemented R2 medium (Table 1); mating after regeneration could therefore have accounted for the results. A possible exception was *S. coelicolor* A3(2) in SCP1$^-$ SCP2$^-$ condition in which recombination in matings occurs at very low frequencies[10].

In studies of mammalian cells[11,12], DMSO has been found to shift the optimal PEG concentration for fusion from 50% to 40%, allowing the use of a less viscous reagent; this advantage, important for high molecular weight PEG, may be marginal with low molecular weight PEG (such as the 1,540 tested here). With SCP1$^-$ SCP2$^-$ mixtures of *S. coelicolor* A3(2), we tested the effect of omitting DMSO, with or without an increase in PEG concentration from the standard 40% to 50%. At 40% PEG, DMSO caused a significant increase in recombination frequency, but this was largely compensated by raising the PEG concentration to 50%. These findings were strikingly comparable with those for mammalian cells Since the standard procedure was consistently as good as or better than the variations on it, we saw no reason to depart from it.

Analysis of the segregation of non-selected markers amongst recombinants from six-factor crosses of *S. coelicolor* A3(2) derivatives (SCP1$^-$ SCP2$^+$ × SCP1$^-$ SCP2$^+$ or SCP1$^-$ SCP2$^-$ × SCP1$^-$ SCP2$^-$) was done by selecting recombinants inheriting markers at two loci on opposite sides of the circular linkage map with two non-selected markers in each half of the linkage map to divide each half into three approximately equal map intervals. In a mating, multiple crossover classes represented about 2% of total progeny, but after protoplast fusion they represented over 20%. This loosening of linkage (increased recombination per unit of map length) might in part reflect a more complete diploidy in the units produced by protoplast fusion than in the merozygotes arising by mating; another possible explanation is multiple rounds of recombination in the large units arising by multiple protoplast fusion and regeneration (an enormous increase in protoplast size was described[5] before regeneration).

The technique described here should be useful in at least some recombinational approaches to industrial strain improvement. Particularly important are the very high recombination frequencies achieved, even in the absence of detectable sex factor activity. a frequency of 6% recombinants inheriting one of two alleles at each of two loci is equivalent to a total 'sexual' progeny of 24%. Moreover, the observed linkage relaxation would lead to a much more nearly random assortment of genotypes in a cross than would normally be the case. Both features would be ideal in the crossing of divergent lines obtained by successive mutant selection in order to generate an assortment of new genotypes, including those in which positive gene interactions gave rise to superior performance, or in which groups of negatively interacting genes had been disrupted[13]. Once recombinant yields had been optimised for a given set of strains, by minimising contamination of protoplasts with non-protoplasted units and by equalising the frequencies of the two parental genotypes, the total non-selected progeny of protoplast fusion could be screened for desirable phenotypes without the need for selectable markers When two parents differ by several mutations, the great majority of 'sexual' progeny are recombinant in respect of these mutations. Even with a comparatively modest total frequency of such progeny of 10%, the percentage of unimproved genotypes in the protoplast fusion procedure could be less than that of unimproved and disadvantageous genotypes which survive a typical mutational step in strain improvement.

Other potential contributions of the procedure to *Streptomyces* genetics would be in the synthesis of genotypes carrying combinations of known markers. A limiting step in the development of genetics in a new strain is the production of multiply-marked stocks by the successive induction of mutations, usually auxotrophs. In matings, two auxotrophic markers are lost by counter-selection whenever two lines are combined and at least three different markers are needed in each parent before recombinants can be selected carrying more markers than either parent. By protoplast fusion, the markers of two parents can be combined without loss, allowing the rapid construction of complex genotypes. Whether or not the technique described here can be applied to the other important genera of actinomycetes, and to interspecific combinations, remains to be seen.

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Test of the ballistic hypothesis of movement and the 'point of no return'

How long does it take to stop or modify a movement once it has begun? Where there is an error in producing an action as intended (usually because of control-display incompatibility) subjects may correct it in less than their normal reaction time (RT) by proprioception[1] or efference-copy monitoring[2–4]. Where subjects work from a visual (external) error signal, however, they are usually said to take a full RT to stop[5] or correct[6,7] ongoing movements, so that there is a 'point of no return', after which they are committed for about 300 ms to any action decided on[8]. During this time the action is ballistic at the perceptual-motor level[8,9,10]. But in a recent tracking experiment[11] where subjects wrongly anticipated the target motion, and so began a movement in the wrong direction, the correction time to stop the action (CorT) was often much shorter than the normal RT to initiate movements, down to as little as 120 ms in some cases. This suggests that fast error correction can occur from a visual signal alone, at least to stop movements (as in an emergency). Repeating the experiment we find that movements can indeed be arrested soon after they begin, but only if the beginning is delayed; it always takes one RT from the occurrence of the error signal. We therefore infer that there is a 'point of no return' at the decision level, but that movements are not necessarily ballistic at the motor level.

The experiment requires subjects to track predictively a target moving in a regular sequence of steps. They are told to try to synchronise their movements with those of the target, and thus to begin their actions at about the time they anticipate it will move. The target is then occasionally made to jump in the direction opposite to that expected, and the time taken to stop wrongly-predicted movements at these 'surprise jumps' (SJs) measured. As control and display are compatible, the only error signal is visual, and CorTs for incorrect movements are directly comparable with both normal RTs and RTs for correct movements at SJs (where subjects do not make errors).

Fifteen students were tested using an oscilloscope display and joystick control (described elsewhere[11]). The 3-mm target circle jumped horizontally from the centre of the screen to points 30 mm to left or right, and back, pausing for about 2 s at outward points and 1 s in the centre. There were seven trials of 20 cycles (120 s) each. On trials 1 and 7 the pattern of jumps outwards from the centre was irregular, and subjects followed the target as quickly as possible. On all other trials the target jumped alternately left and right (except where SJs occurred in trials 4, 5 and 6). On trials 2 and 6 subjects followed the target; on trials 3, 4 and 5 they tried to synchronise their movement with it. RTs and CorTs (for outward movements only) were measured from ultraviolet records to an accuracy of 10 ms.

Table 1 Reaction times for correct movements

Class of movement	Trials	N	Mean (ms)	s.d. (ms)
A Follow irregular pattern	1 and 7	270	211	44
B Follow regular pattern	2 and 6	270	173	70
C Predict regular pattern	3, 4, 5	360	−199	319
D Correct moves to SJs (median for each S)	4, 5, 6	15	214	36

Table 1 lists overall RTs for correct movements in the various conditions. Each estimate is derived from six or eight movements made by each subject in one or two samples taken from the trials listed, except 1*D* where numbers varied between subjects and the median score for each subject was used for the calculation. (1*D* represents RTs for correct movements at SJs.) Within all these classes of movement initial ANOVAs showed that there were no significant differences due to samples, trials or directions of movement. RTs for conditions 1*A* and 1*B* are similar to those found elsewhere[1,10,11]. 1*C* shows that subjects were predicting on trials 3, 4 and 5, since their RTs were mostly negative, and rarely longer than +120 ms.

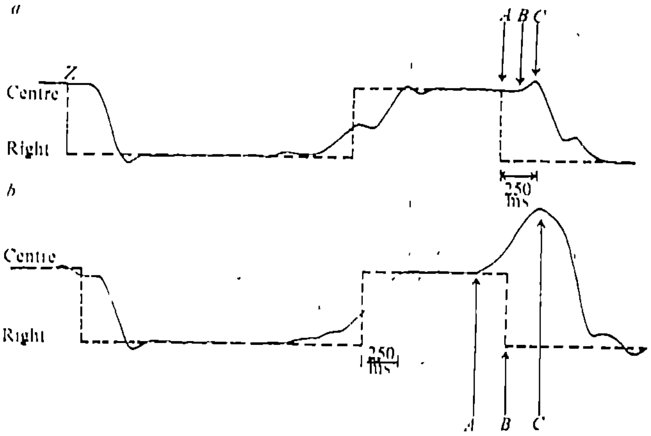

Fig. 1 Record tracing of wrongly-predicted movement. Dotted line, target; continuous line, subject. The track runs from left to right. *a*, At point *Z* the subject makes a normal aimed movement to the right, with an RT somewhat shorter than those to irregular target movements. At *B* he begins a movement to the left (expected direction of target jump) when the target in fact moves to the right again (point *A*). *A*—*B* = RT (140 ms); *B*—*C* = CorT (110 ms); this is much shorter than the average RT to follow the target, so the motor pattern initiated by an order from the voluntary decision mechanism is not completely ballistic once it has begun. It may be shut off during its execution if necessary, but only if it begins some time after the target jumps; the total time RT+CorT is still at least one normal RT. *b*, At *A* the subject commits himself to a movement in what turns out at *B* to be the wrong direction. *A*—*B* = negative 'RT' (−170 ms); *B*—*C* = CorT (230 ms); this is about the same as the subject's normal RT. The CorT *B*—*C* here is about equal to the combined RT and CorT of corrections in *a*. When the subject is committed to his action in advance of the error signal, therefore, CorTs are not shorter than the normal RT, and are often rather longer.

1*D* shows that responses which correctly follow an unexpected direction of movement only do so after a lag of duration similar to the normal RT for irregular jumps 1*A*, that is, when the signal tells the subject which way to go. These RTs are significantly longer than those of 1*B* where the signal tells the subject when to go, the direction already being known ($t = 2.322$, $P < 0.05$ for 15 subjects).

Table 2 lists RTs and CorTs for incorrect predictive movements at SJs. Those begun after the target jumps (Fig. 1*a*) show evidence of relatively fast error correction (180 ms), this being roughly equivalent to RTs for the regular pattern (Table 1*B*), suggesting that a 'stop' order may take only as long as a simple 'go' order. The shortest CorT found in these records was 70 ms, and a dozen or so were less than 100 ms, which is shorter even than the time suggested for a motor system servo to adjust by some internal feedback[13], so there may not be a minimum value of CorT for cutting off a movement in progress. Shorter CorTs than this, however, may be difficult to distinguish from natural tremor, although there was little evidence of the latter in these records—movements started and stopped quite distinctly on most occasions (for example, *Z* in Fig. 1*a*). Very short CorTs only occur, however, where the onset of the action is delayed, and the error signal therefore has time to begin its passage through the central nervous system before the movement starts. When RT and CorT are added together (2*A*, bottom line), correction times again average about 300 ms from the time of the error signal.

Table 2 Reaction times and correction times for errors at SJs

Class of RT/CorT	Trials	*N*	Mean (ms)	s.d. (ms)
A, Incorrect movements with positive RTs: (median for each S)				
—RT	4, 5, and 6	15	143	41
−CorT	4, 5, and 6	15	181	53
−RT+CorT	4, 5, and 6	15	309	52
B, Incorrect movements with negative RTs: (median for each S)				
−RT	4 and 5	15	−231	117
−CorT	4 and 5	15	309	98

Where incorrect movements begin in advance of the target jumping (Fig. 1*b*) subjects need about 310±100 ms to stop an ongoing movement. This is close to the minimum value reported by others for stopping an action[5–7], or adjusting it through vision[14].

These results suggest: (1) fast error-correction can occur from an external (visual) error signal alone (that is when an efference-copy explanation cannot apply); thus (2) movements are not necessarily ballistic in that they may be stopped, if found to be wrong, before they have run their full course. This takes about 180 ms on average, but sometimes only 70 ms or less. It occurs where subjects insert a delay between decision and action which partly bridges the normal RT; but (3) movements cannot be stopped in less than 300 ms from the occurrence of the error signal, that is, visual error correction does conform to the ballistic hypothesis at the highest perceptual-motor 'decision' level; (4) a predictive strategy reduces RT and gives better control where events are redundant, but lays the operator open to predictive error, the duration of which varies inversely with its initial RT. It must last a full RT, if, as in many skills, the action initiated from the prediction runs in time with the events predicted—this is the 'point of no return' of the wartime pilot, batsman or driver in an accident. Subjects working predictively, therefore, trade off a minimum RT for predictable events against the risk of a relatively long CorT for unexpected events when their predictions fail; the payoff values of errors and reactions will presumably determine their strategy in individual cases[1].

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Lateralised perception of bilateral chimaeric faces by normal subjects

A COMPOSITE 'chimaeric' figure is one constructed by abutting two different half-figures along a vertical join. Following surgical section of the pathways connecting the two cerebral hemispheres (the commissures), patients subjectively experience a compelling perceptual 'completion' in their perception of both halves of such figures when presented at the midline of vision. This perceptual completion evidently occurs independently in each of the two cerebral hemispheres[1]. The critical condition for this phenomenon might be the disconnection between the primary visual cortices of the two hemispheres. Only those portions of these cortical areas are cross-connected which represent a narrow strip of the visual field in the region of the vertical midline[2]. We, therefore, tested normal subjects with chimaeric faces in which no vertical join was visible. Our subjects then experienced perceptual completion, as we had predicted.

There are at least three primary areas in the primate cortex which seem to be involved in the preliminary processes of visual pattern analysis, and which are known to be organised retinotopically[3,4]. These three areas, known as V1, V2 and V3, give rise to commissural fibres which pass through the posterior splenium to corresponding areas in the opposite hemisphere. However, the cross-connected points are limited to the portions in each area which represent a strip of the visual half-field some 2–3° wide bordering the vertical meridian[2–8]. One function which some of these fibres could perform is to assist the brain in its alignment of information deriving from the two visual half-fields[9]:

Table 1 Numbers of choices based on each constituent half-face (join visible)*

	Response condition					
	Pointing (right hand)		Pointing (left hand)		Verbal	
Response based on	LHF†	RHF	LHF	RHF	LHF	RHF
Mean	6.92	6.50	9.67	7.00	6.42	5.83
S.D.	1.68	2.88	1.37	2.59	1.00	1.40

* The tabulated scores are based upon all 12 subjects of group A.
† LHF/RHF = left/right half-face.

Table 2 Numbers of choices based on each constituent half-face (joint hidden)*

	Response condition								
	Pointing (right hand)			Pointing (left hand)			Verbal		
Response based on	LHF	RHF	Neither	LHF	RHF	Neither	LHF	RHF	Neither
Mean	4.50	5.00	2 50	7.00	3.13	1.88	2 57	3.43	1 00
S.D.	1.31	1.31	—	1.51	0.83	—	1.51	1.40	—

* The tabulated scores refer only to those subjects of group B who did not spot the stimulus asymmetry during the relevant test condition. There were eight such subjects in the manual response condition, and seven in the verbal response condition.

this might proceed by a constant process of subtractive cross-matching between similar feature-detecting units with correspondingly-placed receptive fields in the two adjacent half-field strips. The use of some such process could account for observations that an immediate impression of symmetry or asymmetry in a dot-pattern is based, other things being equal, on the symmetry or asymmetry of only the middle region of the array[7].

If this argument is valid, then it might be these splenial fibres which enable the normal observer to detect readily the true nature of a centrally-flashed chimaeric figure. If so, then this detection should become very difficult if the central portion of such a figure were concealed; and since then no mis-match would occur, perceptual completion should be experienced.

We used two groups of 12 subjects, undergraduate students aged between 17 and 25. Both groups were tested comparably, viewing a series of chimaeric faces (made from colour photographs), each back-projected for 100 ms on a translucent screen at luminance levels of 30–40 Ft.L. A total of 24 chimaeric faces mixed with 12 normal faces were given first, the subject in each case being required to identify by pointing the face (or faces) he had seen, from amongst a total set of 12 possible faces. For the first half of these trials, the right hand was used in pointing, and for the second half the left hand. Next, a subset of six faces were given names, which the subject memorised. A second series of colour slides was then presented (seven chimaeric, six normal) with the subject in each case naming the face(s) that he had seen. The left/right occurrence of half-faces was balanced either within subjects (pointing condition) or between subjects (verbal condition). With one group of subjects (group A) conventional chimaeric faces were used, whilst with the other (group B), the same photographic originals had been overlaid with a vertical white strip of paper before the final photographic stage. The mean angular size of the faces (ear-to-ear) was 25°, and of the blank strip 5.3°. In all cases, the projected picture was centred on a spot on the screen which the subject was required to fixate.

The subjects of group A soon realised that they were viewing asymmetrical faces (between two and six chimaeric slides being required). Choices were not clearly biased in favour of the left or right half-face, except in the pointing condition where the left hand was used (Table 1). Analysis of variance of the pointing responses reveals a highly significant main effect of hand used ($F_{1,11} = 26.69$, $P < 0.001$), but this is confounded with a possible order effect. However, the significant interaction of hands × half-fields ($F_{1,11} = 9\ 00$, $P < 0.05$) reflects the fact that this improvement with change of hand was restricted to an increase in correct selections based on the left half of vision.

In group B, seven subjects never noticed that asymmetrical faces were being used, and the other five required between 11 and 29 chimaeric stimuli before recognising any asymmetry. Except beyond such a point, all subjects in group B indicated (manually or verbally) only one face on each trial. Subsequent careful questioning confirmed that they had always subjectively perceived a single complete face. There was a tendency for these 'completing' subjects to name the face which had been exposed to the right half-field rather than that to the left, but this bias was not statistically significant (Table 2). However, analysis of variance on the choices made by pointing, showed a significant main effect of half-fields ($F_{1,7} = 7.43$, $P < 0.05$), in favour of the left. In addition there was also a highly significant interaction of hands × half-fields ($F_{1,7} = 38\ 45$, $P < 0.001$), again reflecting the fact that an asymmetrical distribution of choices appeared only when the left hand was being used for pointing.

Preventing normal subjects from seeing the join in chimaeric faces did not make them behave exactly like commissurotomised subjects Nonetheless, perceptual completion did predominate in this condition, and several subjects even denied having seen any blank strip. Furthermore, completion evidently occurred irrespective of whether the half-face that determined response was in the left or right half-field.

We have also found that a girl with callosal agenesis, who is impaired in midline stereopsis[8] (see ref. 9), showed perfect perceptual completion using conventional chimaeric figures (with the two halves directly abutting)[10]. This observation agrees with our present suggestions, if it can be argued that her brain has compensated, perhaps through the anterior commissure[11], for all of those visual cross-connections normally passing through the corpus callosum, except for those of visual areas V1, V2 and V3.

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matters arising

Dysmyelination in Jimpy mouse due to astroglial hyperplasia?

SKOFF[1] recently suggested a new explanation for the central dysmyelination in Jimpy mice. He suggested that astrocytic processes branch abnormally in these mutants before oligodendroglial proliferation. This branching continues until it surrounds most axons. It is further suggested that by this mechanism oligodendrocytes are prevented from reaching the axons, thus leading to secondary inhibition of oligodendroglial development and myelin formation. This concept, however, seems to be inadequately supported by the quantitative and qualitative data given.

The difference in the ratio of astrocyte cytoplasm to optic nerve tissue between hemizygous controls and Jimpys which were calculated in the planimetric study (ref. 1, Table 1) seems to be much too small to support the proposed thesis. A comparison against animals of a different strain is not valid. Considering the absolute cell counts (ref. 1, Table 2) the calculated surface area of six missing, non astrological cells (72 in hemizygotes, 66 in Jimpys) must be taken into account, if the ratio is to be interpreted as an expression of true astrocytic hyperplasia.

No illustration from the premyelination period showing the astroglial processes surrounding the axons is presented. The vast majority of axons in Fig. 2 ref. 1 are not in contact with astrocytic processes. Figure 3 in ref. 1 is irrelevant for Skoff"s argument because it is obtained from optic nerve at day 23, that is, 15 d after onset of myelination. The cited illustrations (ref. 2, Figs 7 and 8) are also irrelevant for the same reason. The effects which Skoff claims are probably concerned with pathologies following the primary event. The concept that reactive astrocytosis follows central nervous system pathology is well established.

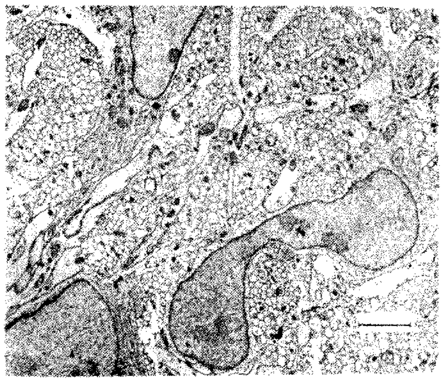

Fig. 1 8-d-old Jimpy mouse optic nerve exhibiting large groups of unmyelinated axons without contact to astroglial processes.

Can astrocytic processes block oligodendrocytes from contacting axons? This hypothesis seems questionable. Blakemore[3-5] produced degeneration of oligodendrocytes, demyelination and heavy astrocytosis in young mice with cuprizone. Demyelinated axons were ensheathed by astrocytic processes. Drug withdrawal led to clear remyelination induced by newly developing oligodendrocytes.

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SKOFF REPLIES,—Meier and Bischoff[1] say that the data used to support the hypothesis[2] that astroglial cells may interfere with myelination is inadequate. They claim that the astrocytic processes in Jimpy optic nerve do not contact the majority of the axons before the onset of myelination and that to show this condition afterwards is irrelevant. In the photograph of an 8-d-old Jimpy mouse optic nerve (Fig. 1 ref. 1), Meier and Bischoff state that large groups of axons are not contacted by astrocytic processes. Careful examination of this picture, however, shows that the large astroglial processes send out very fine branches into the fascicles of axons. I have calculated from the original photograph that 77% of the axons are contacted by astrocytic cytoplasm. At this stage, about 2% of the axons in littermates are in the process of being myelinated[3]. In my own 8-d material from Jimpy optic nerve, I have observed that the astrocytic processes not only contact the majority of the axons but they, in part, surround individual axons; this process continues throughout myelinogenesis[2]. I have demonstrated that the abnormal branching of astrocytic processes in the optic nerve begins 2–3 d before the onset of myelin formation. Since this astrogliosis (increase in processes and filaments) begins before myelinogenesis, it is reasonable to conclude that the astrocytic abnormality is not a reaction to abnormal myelination.

In response to the comments[1] that the ratio of astrocytic cytoplasm is too small to support the astroglial hypothesis, these ratios represent a 39% increase over the Swiss strain and an 18% increase over the heterozygote (the term hemizygote was used in the original paper). Concern over the effect of the missing cells on the above ratios is irrelevant since the area of the astrocytic cytoplasm was directly determined rather than by multiplying the number of astrocytes by an average area. The ratios were included to show that the total amount of astrocytic cytoplasm seems to be increased in electron microscopic preparations. It should be stressed that an increase in glial cytoplasm is not essential for an increase in the branching of their processes (two cells of a given volume can take on entirely different geometric configurations).

The Jimpy mice in this study were always compared with their littermates[2]. The Swiss mouse was used as a control for the entire strain because gliogenesis seems somewhat abnormal in most animals of the litter. Electron microscopic observations of Jimpy littermates show varying degrees of astrogliosis in both optic nerve and spinal cord; the number of axons being myelinated in the branchial spinal cord also varies considerably in littermates of identical body weights.

The model of demyelination using cuprizone[4] is important because it shows that normal animals can remyelinate after the drug has been removed from their diets. But this study does not explain the mechanism of remyelination, that is, whether the astroglial cells first withdraw their processes or whether the oligodendroglial cells displace them. In contrast to this model, most human demyelinating diseases[5-8] exhibit an astroglial reaction but the degree or remyelination is poor to nonexistent.

The earliest detectable morphological abnormality in this study of Jimpy mice lies in the astrocytes. To date, there is neither morphological nor biochemical evidence to suggest that the axons are abnormal at the onset of the astrogliosis. It remains to be determined whether the astrogliosis is a reaction of the astrocyte to the axons or if it is inherent to the glial cell. As regards the relationship between the astrogliosis and myelination, the critical question is not so much the cause of the astrocytic abnormality but whether the condition itself interferes with myelination.

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Nature of stromatoporoids

KAZMIERCZAK[1] argues that the spherulitic microstructure of stromatoporoids, together with the spherulitic infilling of astrorhizal canals in specimens described from the Upper Devonian of south-eastern Poland, indicate that they have been formed by the calcification of coccoid blue-green algal cells. This suggestion, that stromatoporoids are calcified cyanophyte colonies, is novel and stimulating but it is open to two principal objections. First, it fails to consider alternative explanations of the spherulites; secondly, it overlooks a variety of additional complex morphological features of stromatoporoids.

Stromatoporoids have also been compared with coelenterates[2,3] and sponges[4] and representatives of both groups have skeletons composed of aragonitic spherulites. The similarity of this to the granular or melanospheric microfabric of stromatoporoids has been detailed by Stearn[5]. Several non-skeletal origins of the spherule-like infillings of the astrorhizae described by Kazmierczak[1] are possible, including sedimentation of fine peloidal material, cementation, and recrystallisation. However, Kazmierczak does not deal with any of these points but instead assumes that calcification of coccoid cells must be the origin of skeleton and infilling alike.

The characteristic skeletal features of stromatoporoids such as laminae, pillars and canals together with the not uncommon cyst plates, basal layers and external epithecal coverings, appear to have no parallels in cyanophyte colonies. The permineralised cyanophyte colonies[6] cited by Kazmierczak do not resemble stromatoporoids.

Kazmierczak is essentially proposing that stromatoporoids are skeletal stromatolites[7] of the thrombolitic variety[8]. Stromatoporoids and stromatolites, although superficially similar, differ in external morphology, internal structure, and geological distribution. The complex and varied internal morphology and limited geological range of stromatoporoids, together with their tendency toward discrete forms, strongly suggest that they are higher organisms than cyanophytes.

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Do antigen-specific helper factors in rabbit lack V-region Ig determinants?

TAUSSIG, Finch, and Kelus[1] proposed that antigen-specific helper factors derived from T cells in rabbit lack V-region Ig determinants. They further argued that T-cell surface receptor would also lack V-region Ig determinants since the V regions of both the helper factors and T-cell receptor would very likely be the same. Their proposal was based on the finding that sheep red blood cell (SRBC) specific helper factors derived from rabbit carrying an allotypic marker at the a locus in the V region of its immunoglobulins is not adsorbed by anti-allotype antibody (anti-al, Table 2 in ref. 1) conjugated to Sepharose. Since no adsorption occured the authors interpreted it as an indication that the allotypic marker was not present in the SRBC-specific helper factors. In fact, Table 2 in ref. 1 shows that the helper activity is partly adsorbed by anti-al-Sepharose; the number of plaque forming colonies (PFCs) is reduced from 3,200 ± 365 to 2,450 ± 280 as a result of adsorption (Table 2, ref. 1). Since nothing is known about the relationship between PFC and quantity of helper factor used, it is difficult to say anything about the percentage of factor adsorbed by the immunoadsorbent. If the quantity of factor used in the control giving 3,200 ± 365 PFC is far greater than the optimum required (minimum quantity required to give maximum PFC) then the reduction in PFC after passing through the immunoadsorbent observed may be quite significant and represent a substantial reduction in helper activity. But, the authors have not studied the dependence of PFC on quantity of helper factor and not made any attempt to quantitate the factor before and after passing through the immunoadsorbent. It seems to me that the crude data presented do not allow the proposal the authors have made.

If indeed the data show a lack of adsorption of the helper factor by anti-al-Sepharose, then the lack of adsorption can be explained in an alternate way also. Since the helper factor has the capacity to bind antigen and since the factor was generated by incubating lymphocytes with antigen, it is conceivable that the factor is complexed with antigen fragments. These antigen fragments, though non-immunogenic and incapable of sensitising the bone marrow cells, may be large enough to sterically block the allotypic (al) determinant and thus prevent recognition by anti-al antibody. The authors, thus, have not ruled out the possibility that V-region immunoglobulin determinants are present in antigen-specific helper factors and receptor molecules of T helper cells. Whether or not the antigen-recognising sites of T helper cell receptor molecules and the helper factors are controlled by immunoglobulin V genes in rabbit, thus, remains an open question.

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TAUSSIG *et al.* REPLY—Dr Sundharadas has raised certain points concerning removal of the rabbit antigen-specific T-cell factor on immunoadsorbents carrying anti-immunoglobulin (Ig) reagents. The difference in numbers of PFCs which he points out is not significant (at the 5% level). The capacity of the anti-al allotype adsorbent was more than sufficient, on past experience, to have removed the factor had the latter carried the al allotypic marker. As stated in the paper, the method was sensitive to a 50% reduction in factor activity, based on titration. The question is really not whether al-carrying molecules could have escaped detection, but rather whether a proportion of factor molecules greater than 50% could have Ig V regions yet be negative for a allotype, as are about 10% of normal rabbit Ig molecules. This cannot be ruled out, as discussed in our paper. Thus, at the very least our results show that the factor molecules do not carry the Ig allotype in the same proportion as circulating Ig. The further point about possible blocking of the factor binding site and steric hindrance of the allotype we consider unlikely. Evidence against it is that the factor can be absorbed by antigen, indicating the availability of its binding site.

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reviews

Basic modern astrophysics

Paul Davies

The Structure of the Universe. By Jayant Narlikar Pp. 264. (Oxford University: London, Oxford and New York, 1977.) £4.25.

THE name of Jayant Narlikar has long been associated with the steady-state theory of the Universe, the absorber theory of radiation and other controversial aspects of modern cosmology. Now he has used his fertile imagination to produce a readable and useful introductory book to astronomy and cosmology. Although the first half of the book deals in a conventional manner with standard topics such as stellar evolution, galactic structure and Friedmann cosmological models, some of the legacies of the author's more speculative past research emerge in later sections. The chapter entitled The Universe and the Arrow of Time will be familiar to those who have read Professor Narlikar's earlier (much more specialist) book written with Sir Fred Hoyle, although the uninitiated will probably be unable to follow the subleties of the arguments about past and future absorbers, and initiates may well disagree with the conclusions.

The text has obviously been prepared with a view to commanding a wide readership which can range from the scientifically-inclined layman seeking an easy-going summary of this popular subject, up to astronomy and physics undergraduates requiring background or introductory reading. To this end the mathematics has been kept to a minimum, and where possible is separated from the main part of the text in special boxes. There is a liberal distribution of graphs and line drawings, some of which seem to have been prepared hurriedly, and one of which (concerning the Friedmann cosmological models) is badly wrong and could be seriously misleading for a student. In spite of these slips, the open style and lucid presentation generally make information retrieval easy and straightforward.

Superficially, *The Structure of the Universe* seems like a popular book. Its jacket begins with the pregnant query "Do black holes really exist?", and follows up with such imponderables as "what is a quasar really like?" In spite of this, black holes only occupy two or three pages buried in the chapter on gravitation, and most of the discussion about this and other much-exposed topics in modern astronomy is explanatory rather than descriptive.

There is an unmistakable philosophical flavour running through the discussion, with the theme that local physics and global structure are somehow interwoven and that recent developments in both observational and theoretical astronomy are beginning to provide a tantalising glimpse of this link. This is an idea certain to appeal to non-specialist readers who are frequently intrigued by the concept of a grand design, but students would do well to temper enthusiasm for this speculation with healthy scepticism, for much of the physics of the 'micro-macro' link such as the absorber theory of radiation and Mach's principle, is not universally accepted. I was a little disapointed that Professor Narlikar, for so long a spirited advocate of the steady-state theory, has not taken the opportunity to present an extended counterattack in its favour.

In summary, this is a nicely written, attractive book, including many useful, if somewhat superficial, discussions of basic modern astrophysics, from an author of international repute. □

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Receptor research

Methods in Receptor Research, Parts 1 and 2. By M. Blecher. Pp. xiv+383 and 385–763. (Marcel Dekker: New York, 1976.) SFrs. 120 per part.

THESE two volumes have been designed to form a laboratory manual giving a more detailed description of methods used in receptor research than is commonly found in papers published in journals. In this aim, the editor and the 41 authors have succeeded. It was decided to omit the receptors for steroid and thyroid hormones because they have little in common with the polypeptide hormones, which take up most of the space. There are three chapters on gonadotropin receptors, three chapters dealing with glucagon receptors, three chapters covering insulin receptors, two chapters on prostaglandin receptors and one chapter each on β-adrenoceptor and on the receptors for acetylchlorine, adrenocorticotropin, oxytocin, vasopressin, thyrotropin, parathormone, prolactin, cholera toxin, lectin and opiates. Each chapter is written by an expert in the field, describing the laboratory techniques in considerable detail. As with other treatises covering rapidly expanding areas of research, it could not be avoided that there are some notable absentees, as for instance the dopamine receptor and α-adrenoceptor.

The editor stresses in his preface that the overlap between several chapters is more apparent than real. The reviewer finds himself in agreement with this view because only too often seemingly contradictory results obtained by different laboratories are due to minor but important differences in the experimental techniques used.

It is not possible to deal with the various chapters, which are all well and clearly presented. The newcomer to the field of receptor research will find the detailed description of many well tried methods useful. In addition, there are two carefully considered discussions of a more general nature: Catt *et al.* deal with problems encountered in the determination of binding constants and of concentration of receptor, whereas De Meyts presents sections dealing with the physico-chemical parameters determining optimal steady-state binding and its quantitative analysis, the factors of importance for the study of the kinetics of hormone-receptor binding, and the cooperative interactions among receptor sites.

These well produced volumes will be essential for laboratories engaged in receptor research. Unfortunately, the high price will prevent their acquisition by many individual workers.

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Cognitive development

Development of Cognitive Processes. Edited by Vernon Hamilton and Magdalen D. Vernon. Pp. 772. (Academic: London and New York, 1977.) £21.00.

IT was not so long ago that childhood as a distinct state, with its own distinguishing qualities, was not part of the consciousness of our society. Children wore smaller versions of the same clothes as adults, did similar work, and were expected to behave as much like their elders as possible. There were hardly any books either for, or about, children. Judged by the present volume, although the rest of the world has in the meantime discovered childhood in an upsurge of kids' lib., developmental psychology is still in this undeveloped state.

Current psychological models of cognitive processing are based on empirical investigations with adults. In the present book these models seem to have been adopted without questioning their validity for child development. It is therefore not surprising, that most of the conclusions that are reached are of one of two kinds. First, children, from birth or from a very early age, may show certain information handling capacities, which differ little from those of adults. Alternatively, children are much worse than adults on many cognitive operations, though they become better as they get older. This view is expressed in statements such as: "Increasing age brings increasing ability to make cognitive decisions about a task, and to absorb more information"—which is perhaps not too surprising to anyone but developmental psychologists.

Some of the contributors are clearly aware of the shortcomings of this approach. Thus one of the editors urges in her conclusions that investigators should concentrate on the specific qualities and properties of cognitive processes in children. Two introductory, theoretical chapters do not even pretend to a relevance for development, and though in themselves adequate, state nothing which has not been published extensively elsewhere. In spite of the heavy reliance of cognitive models on the analysis of memory functions, which is reflected in these chapters, the book does not even contain an account of the development of memory.

As one might expect from such a miscellaneous collection, quality of style and content varies greatly. The first ranges from barely comprehensible jargon, such as: "It is worth restating, therefore, that human information processing systems may be variably aroused by diverse mechanisms and cognitive events, that they contain dynamically organised goal-seeking programmes, and that they possess an autonomous general capacity and need to continue operations upon inputs and their stores, which elaborate and reorganise their representations long after the event", to the admirable simplicity of statements such as: "We have two eyes and the most natural way to perceive space is through both of them".

There are excellent accounts of perception of form by Vernon, spatial perception by Walk, conceptualisation by Donaldson and language by Cromer. But in spite of the high qualities of these contributions, and notwithstanding the irrelevant, peudoscientific design of the dust jacket, there is little indication in this book that developmental psychology has begun to put forward its own models and theories. These will have to account for the specific qualitative rather than the merely quantitative characteristics of childrens' cognitive processes.

In the meantime, *vivat vivat* Piaget!

Beate Hermelin

Beate Hermelin is a Research Psychologist in the MRC Developmental Psychology Unit, London, UK.

Plant embryogenesis

Experimental Embryogenesis in Vascular Plants. By V. Raghavan. Pp. 603. Academic: London and New York, 1977.) £21.00, $45.65.

IN an era of symposia and other multiauthored volumes, V. Raghavan deserves to be congratulated on producing this fine volume dealing with a very complex subject which has an immense literature. The reader is immediately impressed with the breadth of topics and the depth of treatment that most receive.

The first and longest section of the book deals with the development from egg to embryo. Among the topics covered by chapters are: structure and organisation of the egg, zygote and embryo; biochemical embryogenesis; nutritional aspects of embryogenesis—both physiological and morphological considerations; embryo culture; embryogenesis in cultured ovaries, ovules and seeds; nutrition and metabolism of cultured embryos; extracellular control of morphogenesis; biochemical ontogeny of cultured embryos, and applied aspects of embryo culture. I was especially impressed with this section because of the excellent organisation, lucid writing and relating of various approaches to one another. While most of the information deals with angiosperm embryos, references are made to pteridophyte and gymnosperm embryos when appropriate.

The second section discusses adventive embryogenesis, including the induction of both haploid and diploid embryoids. The author has made these chapters very informative, for he has not merely summarised the literature but has tried to make some sense out of the many factors controlling embryoid formation, showing clearly that more work is necessary before these factors are understood satisfactorily. A similar appeal is made in relation to the low percentage of microspores that give rise to haploid embryoids. The last section deals with seed dormancy and germination and no doubt many readers will disagree with the author's decision to include them. The last chapters, however, are useful in giving a full perspective of embryogenesis. The argument is put forth that dormacy involves a mechanism of preventing embryo growth and that factors regulating the normal growth and development of the embryo should also apply to the induction and release of dormancy. All three sections are written with clarity and usefully illustrated with drawings, micrographs and graphs. Unfortunately some of the electron micrographs are printed too darkly but on the whole the book's illustrations are very informative. An appendix containing the mineral salt composition of 18 different nutrient media used in embryo culture should prove useful for many readers. The 103 pages of references should also be valuable to the reader.

In the introduction Professor Raghavan expresses the hope that the book will bring together some of the scattered embryological literature and present the current and future perspectives of plant embryogenesis. The book not only attains that goal in its comprehensive treatment but undoubtedly this book will be the definitive volume on experimental plant embryogenesis for some time. It is highly recommended for all those interested in this most interesting and important area of plant morphogenesis.

William Newcomb

William Newcomb is a research associate at the University of Guelph, Canada.

Magnetic ions in metals

Magnetic Ions in Metals: A Review of their Study by Electron Spin Resonance. By R. H. Taylor. Pp. + 118 (Taylor and Francis: London, 1977.) £6.

THE first observation of electric spin resonance (ESR) was reported by Zavoisky in 1945. Since that time the ESR spectrum of every transition and rare earth ion has been observed in non-metallic solids in one valence state or another. The ESR spectrum is usually studied in single crystals to elucidate interactions between the magnetic ion and its local environment. As a result almost all features of these spectra are understood at least in terms of a phenomenological spin Hamiltonian. There have been many reviews of the properties of magnetic ions in non-metal host lattices: the important results and theory are collected in the encyclopaedic monograph of Abragam and Bleaney (1970). No similar authoritative volume has appeared on magnetic ions in metals largely because the application of ESR to metals developed rather slowly; thus most of the important work has been done since 1970. The history of this development is well compiled by Dr Taylor in this welcome addition to the literature on magnetic resonance.

Reasons for the tardy growth in this particular area are to some extent lost to non-experts on metals, but metallurgical factors including single crystal growth have been especially important. I would hardly agree with the author that the rather dramatic arousal of interest since about 1970 is attributable to "increasing efficiency of spectrometer systems" or to "increase in spectrometer resolution", both experimental factors which have changed little since 1962. This book is conveniently divided into three major parts. After some introductory remarks, Dr Taylor gives a very good summary of the main appliactions of the ESR method to metals: studies of ground state splittings, the ESR bottleneck, exchange interactions of magnetic ions with conduction electrons, magnetic order in concentrated alloys, magnetic ions in type II superconductors are all discussed. But by far the major part of the monograph is concerned with an experimental review of the spectra of magnetic ions in simple metals, binary alloys and intermetallic compounds. It may come as a surprise to some readers how few ions with well characterised spectra have been observed in metal host lattices. Most published work concerns the S-state ions Mn, Fe, Gd and Eu; far less has been achieved with non S-state ions, only Er, Dy, Yb and Ce having been observed at all. The monograph is completed by a most valuable review of possible growth areas for ESR studies of metallic solids.

The major strength of the book is that it says clearly what has been achieved up to 1975. It could have been improved somewhat by a more substantial discussion of both the folklore of the subject (for example, what systems have been tried but found not to work) and the theoretical background. But in all it is a well produced volume which will be a valuable addition to the bookshelf of both workers in the field and physicists more generally interested in magnetic resonance.

B. Henderson

B. Henderson is Professor of Natural and Experimental Philosophy at Trinity College, Dublin, Ireland.

Microbiological aspects of waste treatment

Treatment of Industrial Effluents. Edited by A. G. Gallely, C. F. Forster and D. A. Stafford. Pp. 378 (Hodder and Stoughton: London, Sydney, Auckland and Toronto, 1977.) £7.95.

THIS book combines the work of twenty-two authors in twenty-one chapters covering aspects of waste treatment from general description of the basic processes to the problems and treatment of specific industrial wastes. The depth of treatment varies from chapter to chapter, as might be expected with such a large number of authors. Some topics are dealt with in considerable detail and the authors in these cases quote numerous references; the chapter on Surfactant Biodegradation in Waste Waters is particularly valuable.

The editors should have avoided such obvious mistakes as on page one, where the statement is made that "activated sludge plants began to be operated by 1900 AD", when a later author in chapter 5 gives the correct date for the development of the process in 1914. There is also no reason in chapter five to substitute the term 'trickle filters' for the professionally accepted terms of either 'trickling filters' or 'biological filtration'. In chapter 4 it is difficult to interpret the phrase in the section on the determination of nitrate and nitrite; "clean waste such as potable supplied".

The title of the book would have been more explicit if it had included the word 'microbiological'. The book is heavily biased to the microbiological aspects of waste treatment, and it would have been improved by including better information on the physical and physicochemical aspects of waste treament and an introduction to the modern concepts of mathematical description of the processes.

Despite these criticisms, the book will be particularly useful to postgraduate students working in the field.

The amount of information presented in the book, which includes over 800 references, represents a very useful addition to the British textbooks available. The book is well produced and very readable and, compared to other similar books, is good value at the price.

P. Coackley

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Agricultural research strategy

Resource Allocation and Productivity in National and International Agricultural Research. Edited by T. M. Arndt, D. G. Dalrymple and V. W. Ruttan. Pp. 617. (University of Minnesota Press : Minneapolis, 1977.) $25.

CAN science be justified in quantitative economic terms? If no convincing reply, one way or the other, has yet been given, some progress has been made towards the economic analysis of one area, namely research carried out to create new agricultural technology. This research is mostly publicly funded and so has identifiable expenditures; moreover, it generates evident benefits, such as increases in crop yields, which can be quantified. It is virtually indisputable that increases in food supply must improve the welfare of mankind.

Griliches's pioneering study of hybrid maize first provided a methodology, and by 1969 it was possible to hold a symposium on *Resource Allocation in Agricultural Research* (ed. W. L. Fishel, University of Minnesota Press, 1971). We now have the Airlie House conference, which takes account of developments since then.

The book consists of 29 papers which were re-written, and edited, after the symposium. This has blunted the sharp edge of controversy, and it might have been interesting to have included one review paper by a devil's advocate. An issue he might have pursued is the gap which exists between theoreticians (especially model builders) and those needing practical techniques.

Even with careful editing, it could hardly be expected that a coherent synthesis of the whole field would emerge, but the papers provide valuable background on several particular aspects. These are: descriptions of agricultural research institutions, especially those for developing countries, economic analysis of national and international agricultural research systems, and methods of planning and organising agricultural research. It may be mentioned that a good account of the conference is available free as a seminar report from the Agricultural Development Council, New York.

Although all the social implications of technical change in agriculture could not be fully explored, the conference has importance as a deliberate attempt at closer collaboration between social and other scientists, an initiative which is to be welcomed.

The main theme was the "social rate of return on agricultural research", a concept deriving from Griliches. It is well known, even notorious, that such calculations lead to very high numbers, but this raises some queries. Why is agricultural research so productive? If so, why do governments not invest more in it? And if subjective planning methods have proved so successful, why seek to replace them by more elaborate, but still untried, methods?

Although a consensus at the conference did not accept it, the suggestion was made that the rate of return is an inappropriate planning indicator, since it implies that there are no constraints on other factors such as land, labour, energy, capital, imports, and so on. Viewed thus, the high rates of return are only artefacts, and the way could then be open for a different and more realistic presentation to governments of the important contribution which agricultural research can make to national economies.

It is possible, therefore, that the Airlie House conference will prove to be a watershed. Griliches's original study has been prolific, but we need to subject the methodology to close scrutiny and to consider developing improved procedures. This could well be the theme for another conference; meanwhile, this volume provides economists and those concerned in agricultural research strategy with a useful source on current thinking.

W. S. Wise

W. S. Wise is Head of the Programmes Section with the Agricultural Research Council, London, UK.

Free-living and parasitic Protozoa

Protozoa. By A. Westphal (in collaboration with H. Muhlpfordt). (Translated from the German by W. H. R. Lumsden and G. A. Targett.) Pp. 325. (Blackie : Glasgow and London, 1976.) £9.90.

WITH a few notable exceptions one rarely finds modern texts which encompass both free-living and parasitic Protozoa. Any new publications in the field are therefore welcomed. This is a book of high technical quality. It is profusely illustrated with delightful line drawings which, combined with a use of clear and varied typeface in the text, produces a book of immediate visual impact.

From the outset the author has imposed well defined limits on the objectives of this book. In the preface he states "it is not intended to be, and cannot be, anything more than an introduction to protozoology". To this end, the book is divided into three sections: the first, which occupies half the text, deals with the taxonomy of the phylum; the second describes the myriad cell organelles found in these organisms; the final section briefly discusses the ecology of the Protozoa.

In the taxonomic section the wealth of illustration excels. Each class is described in the text concisely, although frequently at the expense of clarity. The description is followed by diagrams showing the range of morphology and life cycles in the relevant organisms. This format does however become wearing on the reader when the illustrations are referred to frequently in the text. The inclusion of a systematic key would have increased the practical value of this book.

The description of the cell biology of the Protozoa provides an insight into the amazing complexity of the subcellular morphology achieved in this 'simple' group of organisms. It is unfortunate that the reproduction of the electron micrographs does not match the quality of the line diagrams. Here, as elsewhere in the book, there is a dogmatic tenacity for a scientifically accurate text rather than a simple and readable one. It is inevitable in such a wide ranging introductory book that that author's necessity for generalisation introduces errors in specific instances. Moreover, the description of meiosis and mitosis does not match the standard set by the remainder of the text, and could well have been omitted.

The final chapter of the book, on the Protozoa and their environment, briefly examines the distribution of some common free-living Protozoa and provides a more expansive and highly readable section of the impact of the principle protozoan parasites of man and his domestic animals.

It is inevitable that this not inexpensive book be compared with Grell's *Protozoology*, a book of similar technical layout and scientific interest, which, although much more expensive, covers all aspects in great depth. Although the latter will certainly be the choice of the committed protozoologist, Westphal's text is one that could be considered for many undergraduate applications.

R. E. Sinden

R. E. Sinden is Lecturer in Zoology in the Department of Zoology and Applied Entomology at Imperial College Field Station, Ascot, UK. He is Secretary of the British Section of the Society of Protozoology.

announcements

Appointments

Dr J. H. Steele, to Director of the Woods Hole Oceanographic Institution, October 1977, from his position as Deputy Director of the Marine Laboratory of Agriculture and Fisheries for Scotland.

Dr D. Silverleaf, to Director of Resource Planning, a new post in the Central Electricity Board's recently formed Research Division, from Director of Resource Planning in the South Western Region.

Awards

The French Society of Physics has granted its awards for 1977. The Jean-Ricard Physics Award was received by Roger Balian for his work at the Saclay Centre for Nuclear Studies (Commission for Atomic Energy). Roger Balian is a theoretician of many particle systems and has worked on various subjects from nuclear physics to the physics of condensed matter. He also did research on helium 3 and wave behaviour.

The Jean-Perrin Award for the Popularisation of Science was shared by Robert Clarke and Nicolas Skrotzky from France-Culture.

The Robin Award, generally granted to a physicist for the whole of his work, went to M. P. Cagnac, a specialist in atomic physics teaching at the Paris VI University.

American scientists Seymour Benzer, a biologist, and John Dyson, a physicist, are the 1977 recipients of the Harvey Prize. Seymour Benzer, Professor of Biology at the California Institute of Technology, receives the Harvey Prize in Human Health for his discoveries in molecular and behavioural genetics, and John Dyson, Professor of Physics at the Institute of Advanced Studies, Princeton, receives the Harvey Prize in Science and Technology for his work in the fields of quantum electrodynamics, ferromagnetism, field theory, static mechanics and the stability of matter.

The Institute of Physics, London, and the German Physical Society announce the award of the 1977 Max Born Medal and Prize to Professor W. E. Spear of Dundee University, for his work on charge transport in non-crystalline semiconductors.

The Biochemical Analysis Prize (10,000 DM) donated every two years by Boehringer, Mannheim, BRD, for outstanding work in the field of biochemical instrumentation and analysis. The presentation will take place during the 1978 Analytical Biochemistry conference in Munich from 18 to 21 April. One or several papers concerning one theme, either published or accepted for publication between 1 October 1975 and 30 September 1977, may be sent in triplicate to Professor I. Trautschold, Secretary of the Biochemical Analysis Prize, Medizinische Hochschule Hanover, Karl-Wiechert-Allee 9, 3000 Hannover 61, BRD, by 15 November 1977.

Person to Person

Exchange 3-bedroom house in Mill Valley, Marin County, California, for similar in north Oxford or nearby for one year beginning August/September 1977. Easy commute to San Francisco. Contact David Martin, Jr, Department of Medicine, University of California, San Francisco, California 94143.

Exchange 3-bedroom house easy access Adelaide, Australia, for similar in London area, December 1977 to January 1979. Contact A. Grady, School of Earth Sciences, Flinders University of S.A., Bedford Park, South Australia 5042.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

Meetings

18–20 July, **1977 Summer Computer Simulation Conference,** Chicago, Illinois (G. Graber, Treasurer 1977 SCSC, Applied Dynamics International Inc., 3800 Stone School Road, Ann Arbor, Michigan 48104, USA).

7–12 August, **1st International Congress for the Study of Child Language,** Tokyo, Japan (F. C. C. Peng, International Christian University, 10-2, 3 Chome, Osawa, Mitaka, Tokyo 181, Japan).

23–24 July, **4th ICU Symposium on Sociolinguistics,** Tokyo, Japan (Department of Linguistics, International Christian University, 10-2, 3 Chome, Osawa, Mitaka, Tokyo 181 Tokyo, Japan).

30–31 July, **2nd International Symposium on Pedolinguistics,** Tokyo, Japan (Department of Linguistics, International Christian University, 10-2, 3 Chome, Osawa, Mitaka, Tokyo 181, Japan).

25–28 August, **The Linderstrom–Lang Conference 1977 on Biochemical Control Mechanisms of Respiration at the Molecular and Tissue Level,** Espoo, Finland (Department of Medical Chemistry, University of Helsinki, Sittavuorenpenger 10, SF-00170 Helsinki 17, Finland).

25–30 August, **International Conference on Chemical Education,** Ljubljana, Yugoslavia (International Union of Pure and Applied Chemistry, University of Ljubljana, Department of Chemistry, RCPU, PO Box 18/1, 61001 Ljubljana, Yugoslavia).

26–30 August, **85th Annual Convention of the American Psychological Association,** San Francisco (Information Office, American Psychological Association, 1200 Seventeenth Street, N.W., Washington, DC 20036, USA).

4–17 September, **Training Course on Membrane Biophysics and Biochemistry,** Cambridge, UK (V. L. Lew, Psysiological Laboratory, Downing Street, Cambridge, UK).

8–9 September, **Conference on The Mechanics of Granular Materials,** London, UK (The Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex, UK).

11–24 September, **Summer School—Thermal Neutron Scattering,** Harwell, UK (B. T. M. Willis, MPD/521.2, AERE Harwell, Didcot, Oxon, UK).

19–21 September, **Biennial Polymer Physics Conference,** Shrivenham, UK (Institute of Physics, 47 Belgrave Square, London SW1, UK).

26–29 September, **1st BOC Priestly Conference on Heterogeneous Oxidation,** Leeds, UK (J. T. Cleave, Special Courses Division, Department of Adult Education and Extramural Studies, University of Leeds, Leeds, UK).

26–29 September, **6th Europhysics Conference on Macromolecular Physics, Phase Transitions in Bulk Polymers,** Varna, Bulgaria (M. Mihailov, Bulgarian Academy of Sciences, Central Laboratory for Polymers, EPS Conference, 1113 Sofia, Bulgaria).

29 September, **Symposium on Food Contamination,** Manchester, UK (G. A. Barker, CPC (United Kingdom) Ltd, Trafford Park, Manchester 17, UK).

Reports and Publications

UK & Ireland—May

Science Policy Research Unit. Annual Report 1976. Pp. 68. (Falmer, Brighton: SPRU, University of Sussex, 1977) [25

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Medical Research Council. Review of Dental Research: a Report by the MRC Dental Committee. Pp. 58. (London: MRC, 20 Park Crescent, W1, 1977.) [25

National Radiological Protection Board. NRPB–R58: A Revised Oceanographic Model to Calculate the Limiting Capacity of the Ocean to Accept Radioactive Waste. By G. A. M. Webb and P. D. Grimwood. Pp. 19. (Harwell, Didcot, Oxon: National Radiological Protection Board, 1976.) [25

Conservation and Metallurgical Process Design. By Herbert H. Kellogg. (Thirteenth Sir Julius Wernher Memorial Lecture of the Institution of Mining and Metallurgy.) Pp. 11. (London: The Institution of Mining and Metallurgy, 44 Portland Place, W1, 1977.) [25

The Welfare of Laboratory Animals: Legal, Scientific and Humaine Requirements. (Proceedings of a Symposium organized by The Universities Federation for Animal Welfare, The British Laboratory Animals Veterinary Association, and the Laboratory Animal Science Association, 30th September and 1st October, 1976.) Pp. 131. (Potters Bar, Herts: The Universities Federation for Animal Welfare, 1977.) [45

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Philosophical Transactions of the Royal Society of London: A: Mathematical and Physical Sciences. Vol. 285, No. 1327: The Moon—a New Appraisal from Space Missions and Laboratory Analyses. Pp. 1–606. (London: The Royal Society, 1977.) UK £36.50; Overseas £37.50. [95

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nature

21 July 1977

Recombinant DNA debate three years on

THREE years this week, the recombinant DNA debate burst into the public spotlight with the publication in *Nature* and *Science* of an appeal for scientists worldwide to defer some types of gene-splicing experiments until their potential public health hazards had been examined. That appeal touched off an extraordinary series of regulatory battles in the United States, and the safety rules governing recombinant DNA experiments have yet to be put into final form. In Europe, meanwhile, most countries have adopted regulatory machinery with very little public interest being expressed in the issues.

In the United States, the matter now rests squarely with the US Congress, with separate bills to regulate recombinant DNA experiments working their way slowly through the Senate and the House of Representatives. The bills have become the focus of a fierce lobbying effort by scientists, and charges of 'Lysenkoism' and political control of basic research are reverberating around Capitol Hill. Though the charges are frequently exaggerated, there is indeed cause for alarm over some aspects of the pending legislation.

The facts are as follows. Last year, the National Institutes of Health (NIH) published a set of guidelines governing recombinant DNA research supported by government funds. The guidelines do not cover research sponsored by industry, however, and they are not backed by inspection or enforcement machinery. Legislation is required to correct those deficiencies, and thus Congress has every reason to be involved in the process. Instead of simply writing the NIH guidelines into legally enforceable regulations, however, Congress is likely to establish complex machinery which could introduce vast amounts of red tape into the support of genetic research. Hence the agitation by scientists.

The agitation is given extra force by the fact that information generated during the past three years indicates that the potential hazards associated with gene-splicing experiments may be more remote than once believed. For example, a special meeting of scientists and health experts, convened by NIH earlier this month, concluded that there is virtually no chance that recombinant DNA experiments could touch off an uncontrollable epidemic. Another good indication of that attitude is the following extract from a lengthy and cogent report on the ecology of *E. coli,* written earlier this year by Dr Roy Curtiss, a respected microbiologist from the University of Alabama who was among the early leaders in calling for strict controls on recombinant DNA research. " I have gradually come to the realisation that the introduction of foreign DNA into EK1 and EK2 host-vectors offers no danger whatsoever to any human being", Curtiss wrote. Thus, while the possibility of hazard seems to be receding, scientists are being faced with increasingly complex regulations.

Most of the complaints have been directed at a bill now before the Senate, written almost entirely by the staff of Senator Edward Kennedy's health subcommittee, but with Kennedy's strong support. The legislation has already been passed by the Senate Committee on Human Resources and is likely to be considered by the full Senate later this month. A separate bill is under consideration by the House Commerce Committee and is expected to be debated by the House of Representatives later this summer.

The two bills differ significantly in their approach. The Kennedy version would establish an eleven-member Presidential Commission to draft new regulations, license facilities and approve individual experiments The House version would require the Secretary of Health, Education and Welfare to draft new regulations, but the regulations would mostly be enforced by local biohazards committees.

The problem with the Kennedy approach is that it would establish a potentially unwieldy bureaucratic arrangement which would require researchers to obtain prior approval for their experiments from a high-level commission. Such arrangements are at present reserved for such matters as the regulation of nuclear power. The House approach offers more flexibility but without any loss of vigilance. In fact, having local committees oversee implementation of regulations is likely to be more effective than vesting such responsibility in the hands of a remote body based in Washington.

So far, the Kennedy bill has been moving through the Senate with alarmingly little discussion. There was no debate at all on the central provisions of the bill at the committee meeting when the legislation was approved, and the only forum for discussion now is the floor of the Senate, which is not the best place for deciding such complex issues. By contrast, the House version was extensively debated by Congressmen during three committee meetings, and it represents a reasonable compromise between political and scientific concerns.

At least the cries of alarm which have been coming from the research community have focused attention on the shortcomings in the Senate bill and there is now some chance that the House approach will ultimately prevail. Nevertheless, the scientists who have been charging 'Lysenkoism' and urging Congress to keep its nose out of basic research should be careful not to damage their case by overstating it. □

A world fit for whales?

Jon Barzdo, Wildlife Consultant to Friends of the Earth in Britain, asks whether the scientific basis for the management of whales through the International Whaling Commission is no more than a facade for the commercial interests of its member countries

WHEN the International Convention for the Regulation of Whaling established the International Whaling Commission (IWC) in 1946 to 'safeguard for future generations the great natural resources represented by the whale stocks' it was naturally heralded as a great advance in the conservation of whales. Indeed it was an advance, as there had been no body controlling the fishery of whales until that time; gray and right whales were given immediate protection, so low were their numbers.

The management system initially used by the IWC classified whales by the blue whale unit (BWu)—a measure of the amount of oil they produced. (1 BWu = 1 blue or 2 fin or 2½ humpback or sei whales.) Thus the system was mathematically rather than biologically based. During its use, blue and humpback whales were fished to commercial extinction. Having reached 6% and 7% of their respective initial populations both species are now protected. In 1963 a Scientific Committee of the IWC recommended the abolition of the BWu. It was abolished nine years later.

Quotas were then set for separate species in different areas, and the maximum sustainable yield (MSY) concept, introduced as the basis for management in 1974 under the 'new management procedure', upheld this system. The principle is to harvest the whales at a level (MSY level) which allows the greatest number of animals to be taken while maintaining a steady population. This is estimated to be about 50% of original population. The intention is to maintain a balance through a higher birth rate and greater availability of food. But this does not allow for competition with other species for krill. Populations of fur and crabeater seal and of penguins both appear to have flourished with the depletion of whale stocks; and we do not know the consequences of man's currently experimental harvesting of krill.

Nor does MSY allow for fluctuations in whale population, or changes in social structure through whale harvesting, or variations in the physical and biological environment (temperature, weather, food supply and so on). Discussing risks associated with different management strategies in the harvesting of baleen whales, J. R. Beddington has shown that as a population approaches MSY level it becomes increasingly unstable, increasingly vulnerable to deleterious perturbation.

Under the 'new management procedure' using MSY, over-harvesting has been such that the IWC has had cause to set zero quotas for fin and Bryde's whales in the southern hemisphere and for fin and sei whales in the north Pacific. For at least five years, and in spite of annual reductions in overall catch quotas, the whalers have consistently failed to reach their quota. Ostensibly as a concession to whaling countries, limits for some species have been regularly set above the previous year's catch level. Not surprisingly, these were never attained. In 1972 the overall quota was 38,600 whales; last year it was set at 27,939. Where do we go from here?

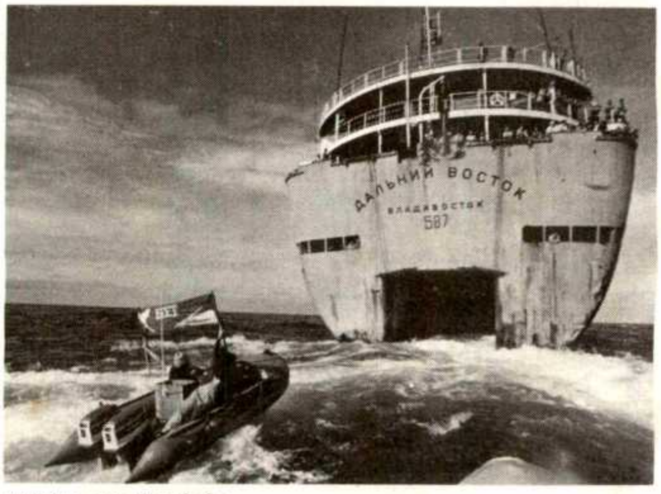
The IWC met in Canberra last month. It made the largest cut in quotas in its 31-year history, a reduction of 10,000 from 1976. The cuts reflect the excessively high quotas of previous years. For minke whales the southern hemisphere reduction of over 300 to 5,690 is a result of the profound lack of data—a problem across the range, and one that increases the difficulty of estimating MSY.

The sperm whale is the most heavily taken species. The world catch limit is 7,356 although it was 23,000 only three years ago, and males have this year been offered complete protection in the north Pacific, though this limit may be revised in November. Britain imports some 8½ million kg of sperm oil (from the creature's head) annually for use in soft fashion leathers and some gear oils. The cost of the oil has been rising fast, not least because the large whales have already been fished out, and the price can reasonably be expected to soar. The leather industry might even start to use some of the range of alternatives that are available.

In 1972 the UN Conference on the Environment in Stockholm called for a 10-year moratorium on commercial whaling. The IWC rejected the call, which is supported by Friends of the Earth (FoE), the International Union for the Conservation of Nature and other conservation bodies. It would allow time for stocks to start recovering, for research into the ecology and populations of whales, and for research into a more efficient and less cruel method of killing whales than that used now.

The moratorium must go on the agenda of next year's IWC meeting. But even if it was approved it is possible that it would not be enforced. For any of the IWC's 16 member nations may legally opt out of any decision of a commission meeting within 90 days; the opting-out procedure is well used and demonstrative of the weak nature of the IWC. In addition, recommendations of its scientific committees have frequently been ignored, notably in 1960 over recommendations regarding blue and humpback whales, in 1962 over quotas and in several years up to 1972 over the use of the BWu. Moreover, there is not a single species whose yield has been sustained under the 'new management procedure'. Most are decidedly diminished.

The International Whaling Commission lacks control and effectiveness and is long overdue for replacement. To rectify this FoE propose the formulation of a UN body to manage the resources of the sea with regard to the complexity of that ecosystem, and for the general good rather than purely that of those with the (albeit ageing) technology to exploit it. ☐



Greenpeace

Fighting over the whale: Greenpeace vs *the USSR*

When two academies meet

Peter Pockley in Sydney outlines attempts by Australian science academies to encourage innovation

TWO learned academies in Australia are putting their weight behind a drive to educate the government and the public in the need for a higher level of local innovation in science and technology. The academies involved are the well-established Australian Academy of Science and the fledgling Australian Academy of Technological Sciences. Both have published booklets recently which catalogue and analyse the R&D scene in Australia and which advocate, *implicitly*, increased government initiative in this area of industry*.

Australian industry is frequently attacked for lack of concern for local innovation and for preferring instead to import new ideas. The number of research staff employed in companies with R&D capacity has dropped sharply, by an estimated 40% in the two years 1974–76. Sums invested in R&D by industry itself, though harder to quantify, are believed to have decreased significantly. Dollars from the government for direct support of R&D in industry, through an Industrial R&D Grants scheme for example, have levelled off. And indirect government support, for example through a tax incentive scheme on industry's own investment in R&D, has not been forthcoming.

Although the extent of overseas ownership of Australia industry has been a hot political issue in the 1970s, the accusation that companies controlled from overseas prefer to import their innovations is generalised largely from the vehicle industry; US-owned car companies reported no Australian innovations in 1970–75. UK- and US-owned chemical industry, by contrast, shows a high rate of innovation within Australia. And some of Australia's more dramatic innovations of late have come from government-financed laboratories, notably in CSIRO, which have developed a successful collaboration with local industry. The present Committee of Inquiry into CSIRO is charged with recommending ways and means of facilitating further collaboration.

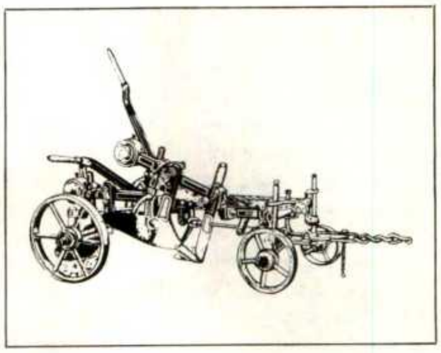
Academy's Forum

The Academy of Science recognised long ago the need for closer liaison with industrialists. Ten years ago it established a Science and Industry Forum with equal membership of academicians (largely basic scientists but including a few with industrial experience) and representatives of industry (businessmen as well as technologists). A pretty informal group holding weekend discussions twice yearly, the Forum has eschewed making recommendations to government but has always managed to attract responsible Ministers and Heads of Departments to its meetings.

Its recently-published set of papers on innovation, under a catchy title and with a commercial distribution and sale system, reflects the Academy's more positive, public face. In the book Sir Alan Walsh, recently knighted for services to science, avers:

> To my mind there are two obstacles to invention and innovation in Australia. The first has its roots in intellectual snobbery which results in many scientists believing that any type of scientific activity which has some real bearing on industry is intellectually and socially inferior to so-called pure research . . . but probably the most serious obstacle is the widspread lack of faith in Australia's ability to generate inventions and innovations which can withstand competition from overseas. In view of Australia's record to date, this attitude is quite ridiculous . . .

The Australian industrialist is more cautious. BHP, Australia's largest locally-owned company, has a good record in Australian-based research and development. Yet its chairman, Sir Ian McLennan, believes there needs to be a "very good reason to commit support to a local invention if the related aims can be satisfactorily met from an available overseas technical development which may be purchased. As there will always be a limit to funds available to develop local innovation these are better spent in a narrower range of likely successes than used over a wide range of relatively ineffectual help . . . " His comments illustrate a growing consciousness in Australia that it is time to formulate and pursue clear national policies on the encouragement of local innovation.



Australia's early innovation: the stump-jump plough

MAAS, Sydney

The Academy of Science is a going force in Australian science, and by far the strongest. With a permanent secretariat, though modestly sized by international standards, it is able to run a range of services to science which goes far wider than keeping happy the exclusive club of its Fellowship of 183 men and two women. The visible presence of its pleasing 'igloo' building on the boundary of the Australian National University has been a significant factor in its influence. Very much a national body, it locks naturally into the bureaucratic, political and academic ethos which dominates the totally non-industrial capital of the nation.

This may have been one of the factors behind a mild blizzard around the Academy's igloo in the 1960s and early 1970s, when a gentle argument about recognition of the applied sciences built up a vortex of discontent. The storm blew around the igloo, only the mildest of breezes being felt within. One of the non-Academician activists was Dr W. A. S. Butement, a former Chief Defence Scientist. He had been leading a move by an informal group dissatisfied with the Academy's terms of admission to Fellowship; these emphasise, as the prime criterion, traditional academic achievement as perceived by peer group elections. The dissenting outsiders tried to persuade the Academy to broaden its terms to include "scientists, engineers and technologists of real worth, who are concerned with the application of science".

Constrained by the terms of its Royal charter, the Academy stuck to its chosen path. Shortly afterwards, however, it did admit to Fellowship some industrially employed scientists. As well as enjoying high standing in the hierarchy of industry, these were men with substantial publications to their name which satisfied the traditional criteria for election of basic scientists. The great majority of technologists employed in industry, universities and government departments were anonymous people by comparison. Thus began a concerted move, centred on an existing, loosely-structured forum of industrial research managers called the Australian Industrial Research Group, for a separate technological academy.

Technological Academy

The Australian Academy of Technological Sciences was formally inaugurated in February 1976 under the Presidency of Sir Ian McLennan.

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* *From Stump-Jump Plough to Interscan* (Australian Academy of Science, PO Box 216, Civic Square, Canberra, ACT 2608).
Sir Ian McLennan, *Innovation in Australian Technology 1970–1975* (Australian Academy of Technological Sciences, Clunies Ross House, 191 Royal Parade, Parkville, Victoria 3052).

Symbolically, the function occurred in Melbourne, one of the nation's two large industrial and financial capitals; the foundation Fellowship was of 64 men and two women. The earlier rift with the Academy of Science was healed, and cooperation was ensured by the presence of 12 Fellows of the Australian Academy of Science. Criteria for election to Fellowship of the new Academy emphasise innovation, but giving to the achievements of its Fellows a collective, collegiate character is a challenge the Academy has not yet solved.

The "suitable safeguards" surrounding government or industrial documents which involve public reticence on the part of its Fellows put the applied Academy at something of a disadvantage in the public prestige stakes when compared with the freer Academy of Science. The technologists have one potential advantage in coming largely from well-heeled industries which should need little persuading to fork out financial support. But a year-and-a-half after inauguration, the new Academy is still walking along rather than jogging. In its first eight months of operation to mid-1976, it spent a mere $4,000. It now maintains a part-time office in Melbourne, but is run on a spare-time basis by busy men. It badly needs to establish a permanent secretariat to service its present needs and future ambitions.

Australian Information Service, London

AAS's igloo

These ambitions are presently centred on the promotion of innovation in industry and government, and on the mounting of a series of annual conferences on the general theme of "Energy and Resources to 2000". The first of these, scheduled for later this year, is on "Fuel and Energy Resources", followed by "Water and Land Resources" in 1978, "Mineral Resources" in 1979, and "Marine Resources" in 1980. And of course a start has been made in publishing the first results of a review of innovative technological achievement in Australia.

One hundred years ago this year, Australia's first industrially successful innovation was registered—the stump-jump plough. It opened up vast tracts of otherwise useless land to cultivation. This year sees a number of internationally competitive innovations beginning to get public recognition. Successful developments, such as the compact Self-Twist Spinning Machine, the Sirotherm process for desalination by thermally regenerated resins, and the Interscan system for aircraft landing control in three dimensions, may begin to persuade government and industry alike that the two Academies are speaking economic sense when they urge more active support for locally based R&D. ☐

Making paper work

Christine Thomas explains the advantages in using recycled and mechanical pulp papers

FEW people are aware, when they purchase good quality writing paper in preference to rougher note paper, or choose extra-soft toilet tissue rather than lower quality crepe tissue paper, that they are contributing to increasing environmental pollution. But investigation of the demand upon resources and the polluting effects of specific processes involved in paper production highlights the considerable differences in the environmental impact of different papers. The general picture that emerges is that the higher the quality of the paper produced, the greater the resource demand and pollution load created.

Paper consists of a network of fibrillated cellulose fibres bonded together by their gelatinous surfaces. The fibres are released from their plant structure during pulping, which for timber can be either by chemical or mechanical means. Chemical pulping methods use chemical solutions to dissolve away part of the plant structure, leaving the cellulose fibres whole and separated with minimum physical damage. Chemical pulp-based papers are considered for many purposes to be superior in quality to mechanical pulp-based papers. Mechanical pulps are generally weaker than chemical pulps and suffer from colour darkening.

Mechanical pulp papers, however, are of adequate quality for many uses, including newsprint, low grade printing papers, fibreboard, wrapping papers, and some tissues. And mechanical pulping provides a more efficient use of timber with a 90–95% yield of pulp from cut, dried wood compared to 40–65% for chemical pulping. Put another way, where it takes on average 13 trees to produce a tonne of chemical pulp, only six would be needed for mechanical pulp. And it is not only the resource demands of chemical pulp which are greater. Its production also creates a significantly more polluting effluent, and consumes more water than does mechanical pulping (see Table 1).

Waste paper can be recycled by repulping and releasing the cellulose fibres from their existing network to allow them to be reformed into a new sheet. Naturally the substitution of recycled papers for those containing virgin pulp will result in a reduction in demand for wood pulp, and will lessen the problems of solid waste disposal. In addition, however, it will result in a reduction of energy demand, of the order of 50% of the energy required for the wood pulping process. It may also lead to less overall pollution, and a saving in water (see Table 2).

Recycled papers themselves vary considerably. Because the fibres have previously been prepared for papermaking, the recycling process in theory is simpler than pulping wood. In practice, though, there are complications introduced by the presence of ink and

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Christine Thomas, author of The Paper Chain, *is a researcher at Earth Resources Research Ltd, London* contraries in the waste paper and by fibre damage due to repeated pulping. The highest grades of waste paper will produce high quality recycled papers with little or no upgrading and will create little environmental impact.

Most waste paper potentially available for recycling, however, requires some upgrading, and here the final product's quality becomes particularly relevant to the environmental impact. A bright, relatively high quality paper, made from low grade waste paper and requiring the fibres to be deinked, will consume more energy and water and will create more pollution than a lower quality paper produced from the same waste furnish. Hence, although an increase in recycling will reduce the overall environmental impact of paper manufacture, this reduction will be smaller if present high quality standards are maintained.

Encouraging recycling

How might attitudes towards paper quality be changed to encourage preferential use of recycled and mechanical pulp papers? The paper industry's resistance to using recycled fibres in some products is partly due to the resultant reduction in quality, which they maintain would not be acceptable to consumers. Whether the consumer or the paper industry needs re-educating, the government could take a positive lead by issuing advice and directives on paper quality standards to its stationery office, to local government and to government departments. This would both encourage a change in attitudes to paper quality and stimulate demand for recycled papers, thereby helping the paper industry to use more waste paper in more of its products.

Such a move could not be expected on environmental grounds alone; but there are strong economic reasons for recycling more waste paper. In 1974 Britain consumed 8.7 million tonnes of paper and board products, of which 47% was imported. Of the remaining 4.6 million tonnes produced in Britain, 45% of the furnish was from imported wood pulp. These pulp and paper imports cost Britain over £1,000 million in that year.

The British paper and board industry's heavy reliance on imported wood pulp puts it at a considerable competitive disadvantage to integrated pulp and paper industries abroad, and this has resulted in increasing dependence on imports of finished goods. In 1960, 77% of all paper and board consumed in Britain was home produced, but by 1974 this figure had dropped to 53%. The industry has suffered contraction and decline. Since 1965 over 30 mills have closed and employment in the industry has dropped by about one-third.

In the face of economic necessity the paper industry is slowly increasing its capacity to recycle. There are considerable obstacles facing a significant increase in waste paper use. Acceptance of lower quality is only one. Plant modifications and availability of waste paper supplies are equally, if not more, important problems to overcome. The government, recognising the former problem, allocated last June £23 million for grant aid towards capital investment for increasing the use of indigenous fibres. With only £6.5 million taken up a year later both the effectiveness of the scheme and the industry's commitment to a significant expansion of recycling are open to question.

The dominant problem in discussions of waste paper recycling concerns its collection. Potentially the waste paper is there, as Britain currently recycles only 27% of its total consumption. But instability in the market has severely weakened the collection network.

To overcome this problem, much of the effort has been focused, unsuccessfully, on stabilising the market. The tasks of building up the capacity to recycle and changing attitudes to paper quality are as important. Indeed, the strong interdependence of these three factors means they must be tackled together. □

Table 1 Average fresh water demand and discharge of pollution from pulping and papermaking in Sweden, 1970-71

	Fresh water demand (m^3/m tonne)	BOD_7[1]	Water pollution Colour	Water pollution Solids	Air pollution[2] (kg/m tonne)
Sulphate pulping[3]	250	50	210	30	20
Sulphite pulping[3]	375	90	85	40	50
Mechanical pulping[4]	50	15	—	20	0
Papermaking	130	—	—	50	0

[1] Discharge of organic matter is measured by Biological Oxygen Demand (BOD_7 in kg/tonne product) and colour (platinum units in kg/tonne product). Figures include contributions from accidental discharges.

[2] Sulphur emissions calculated as sulphur dioxide. Sulphur emissions from oil-fired boilers not included.

[3] Bleached softwood.

[4] Unbleached stone groundwood.

Source: "Pollution Control in the Swedish Pulp and Paper Industry", H. Norrstrom, *Ambio*, **4,** No. 2, 1975.

Table 2 Environmental impact comparison for 1,000 tonnes of pulp

	Low grade paper		Bleached pulp	
	unbleached virgin kraft pulp	recycled fibre	bleached virgin kraft pulp	deinked recycled fibres
Process water use	100×10^6 l	42×10^6 l	195×10^6 l	$16610\times^6$ l
Energy consumption	17×10^{12} J	5×10^{12} J	25×10^{12} J	9×10^{12} J
Air pollutants	42 t	11 t	49 t	20 t
Waterborne wastes emitted (BOD)	15 t	9 t	23 t	20 t
Waterborne wastes emitted (suspended solids)	8 t	6 t	24 t	77 t

Source: Grace and Turner, "Paper recycling and social policy", *Resources Policy*, Dec. 1976

Camera Press

Recycling awaited

Presidential pardon

President Carter's pruning of the White House bureaucracy has spared two science-related offices. Colin Norman reports from Washington

PRESIDENT Carter last week took the first and, he said, "for me the most difficult" step in carrying out his oft-repeated promise to slim down and streamline the federal bureaucracy. He announced a major reorganisation of the White House and the Executive Office, including the abolition of seven offices and the eventual elimination of about 250 jobs, a 15% reduction from present staff levels. In spite of early indications that the fledgling Office of Science and Technology Policy (OSTP) and the Council on Environmental Quality (CEQ) would be among the casualties, both have been spared. They will, however, be required to play their part in the weight reduction programme, and they will lose a few responsibilities.

The overall plan is being billed as a move away from the 'Imperial Presidency' and an attempt to strengthen the role of Cabinet members in formulating Administration policy. Declaring himself "very much opposed" to having the White House in total control of government agencies, with reference to the Nixon White House left unstated but clearly implied, Carter underlined the political importance he attaches to the reorganisation by announcing the plan personally at a packed press conference.

In fact, the offices slated for extinction had mostly been grafted on to the White House by Congress, and their elimination will not be too fiercely resisted. They include the Office of Drug Abuse Policy, the Council on International Economic Policy, the Office of Telecommunications Policy, and the Federal Property Council. The real power centres, such as the National Security Council, the Office of Management and Budget and the Council of Economic Advisers have emerged relatively unscathed. It should also be noted that the White House and Executive Office staff has in fact grown during the first five months of the Carter Administration, from 1,655 to 1,712; the proposed reorganisation will slim it down to 1,459.

As for OSTP, it has come out of the reorganisation in a surprisingly strong position. Though the plan would reduce OSTP's staff from the 32 authorised by Congress to 22, the office would also lose some time-consuming functions, such as the preparation of an annual report on the state of science and technology in the United States. Moreover, since OSTP is still getting under way, it had not filled all of its allotted positions; the staff 'cut' will in fact hold the office to about its present size.

Frank Press, President Carter's science adviser and Director of OSTP, told *Nature* last week that he thinks the plan "looks very good", noting that he has retained all of the central functions of the science adviser's office.

The plan would do away with the President's Committee on Science and Technology, a panel established by the legislation which set up OSTP. The committee was supposed to conduct a two-year review of federal support for science and technology, and recommend changes in the government bureaucracy dealing with research and development. Since Carter has a team of his own looking at government reorganisation, the committee's responsibilities will be taken over by that team and OSTP will be consulted. Committee members were in fact appointed late in the Ford Administration but they have never been reappointed by President Carter, and thus the committee had already informally been scrapped.

Two other important science and technology committees will also have their responsibilities changed under the plan. They are the Federal Coordinating Council for Science, Engineering and Technology (FCCSET), which consists of government officials from agencies which have responsibility for R&D, and the Intergovernmental Science, Engineering and Technology Advisory Panel, a committee chaired by the science adviser which is designed to provide a link between federal R&D programmes and state and local officials. FCCSET will become a sub-cabinet working group, chaired by the President's science adviser, and it will retain its responsibility for coordinating federal research efforts which span several agencies. The fate of the intergovernmental panel is still being worked out, but Press said last week

Debating the neutron bomb

AFTER a long and at times bitter debate, the Senate last week voted to approve production funds for the so-called neutron bomb—a small nuclear weapon designed to emit withering amounts of radiation but relatively low levels of heat and blast. The outcome of the debate was assured earlier in the week, when President Carter personally asked Congress to approve the funds. The House has yet to act on the matter, however, and Carter has also said that he will not decide whether to go ahead with production of the weapon until he has given it further study, but last week's vote is regarded as the crucial test.

The vote was 58 to 38, with several Senators who had originally expressed opposition to the weapon voting for it after Carter had made his feelings known. The Senate later approved an amendment, however, that would give Congress 45 days to pass a resolution disapproving production of the weapons if Carter eventually decides to go ahead with them.

Perhaps the most surprising aspect of the debate about the weapons is that it nearly did not take place at all. The bill containing funds for the production of 'enhanced rediation' weapons, as warheads for the Lance short-range missile, was winding its way through Congress and had progressed a long way before the matter came to public attention. Even President Carter admitted during his press conference last week that he didn't know that the funds were in the bill until he read about it in the newspapers.

The weapons are believed to be small fission–fusion devices which would be exploded at relatively high altitudes over enemy troops. The blast area would be limited to a small region immediately beneath the detonation, but neutrons and gamma rays emitted by the explosion would be distributed over a wider area. The idea, as proponents of the bomb have been quick to point out, is that the device would kill people while leaving most of the buildings in the area standing. But it is an extension of past weapons design, rather than a new weapon.

Proponents of the weapon have pointed out that since it would not cause massive property destruction or contaminate large areas with radioactive fallout, it would provide a more useful and credible battlefield weapon than the present, more destructive, nuclear devices. And that is precisely why the weapon has stirred so much opposition—opponents point out that it would lower the nuclear threshold by blurring the distinction between nuclear and conventional warfare. Herbert Scoville, a former top official in the Central Intelligence Agency, wrote in the *New York Times* last week, for example, that "our security depends on strengthening, not breaking, the barrier between nuclear and conventional conflicts".

Colin Norman

that its functions will be retained in some form.

At one stage, it was widely rumoured that the science adviser would be made part of a policy planning unit in the White House and that OSTP would be scrapped as a separate entity. Such a plan was in fact under consideration by the team which planned the reorganisation, but the team changed its mind and recommended that OSTP be retained. "They came to us as disbelievers but walked away believers", one OSTP official remarked last week.

Though the change of mind may have been brought about by the quality of OSTP's contributions to Presidential decision-making (the explanation offered by OSTP officials), an equally important factor is that OSTP has powerful support from the scientific community and from some influential members of Congress. When President Nixon scrapped the former Office of Science Policy, his action was greeted with a cry of alarm from a number of prominent scientists, including a committee of the National Academy of Sciences. A similar outpouring of wrath would have been certain if Carter had tried to eliminate OSTP. He would also have faced resistance from Congress—which would have to approve such a plan—and in particular from Senator Edward Kennedy, who was the chief sponsor of the legislation which established OSTP.

Similar considerations also led to the reprieve of the Council on Environmental Quality (CEQ), an agency in the Executive Office which is required to monitor compliance by other government agencies with the National Environmental Policy Act, and to provide advice to the President on long-term environmental issues. The reorganisation team was seriously considering scrapping CEQ or at least stripping it of some of its major responsibilities, but when those possibilities were leaked early in July, a hue and cry arose from environmentalists. CEQ has long been regarded as a powerful counterweight to federal agencies which conduct large public works or energy programmes likely to affect the environment, and potential demise would have been regarded as a serious blow to the environmental movement, many of whose leaders were quick to remind Carter that they had helped elect him.

In the end, CEQ will be retained, though its staff will be reduced from 40 to 32, and it will continue to monitor environmental impact statements filed by other government agencies.

By preserving CEQ and OSTP, the reorganisation plan has by-passed two potential sources of trouble in Congress, and the plan should be approved without too much of a fight. ☐

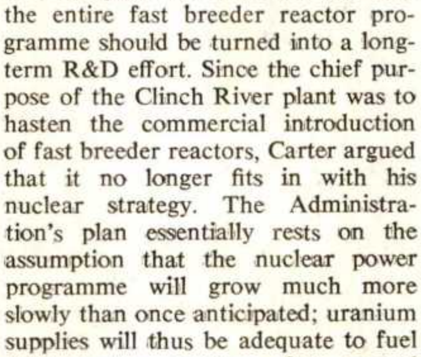
Breeder: far from dead?

PRESIDENT Carter's plans to deter the use of plutonium as a reactor fuel worldwide received a setback last week when the Senate voted to keep the controversial Clinch River Breeder Reactor alive. The move, which came after several hours of intense debate, will not necessarily derail Carter's policy completely, however, for the Senate at least agreed that the project should be deferred for a year, and the House of Representatives has yet to act on the matter. Nevertheless, the vote is being regarded by antinuclear groups as a serious blow.

The Clinch River plant is intended to be a demonstration liquid metal fast breeder reactor, designed to test the commercial feasibility of the fast breeder design. Once expected to cost less than $1,000 million and to be in operation by the late 1970s, its estimated cost has soared to more than $2,000 million and its expected completion date has slipped to at least 1983.

In April, as part of his nuclear nonproliferation policy, Carter proposed that the plant be cancelled and that the entire fast breeder reactor programme should be turned into a long-term R&D effort. Since the chief purpose of the Clinch River plant was to hasten the commercial introduction of fast breeder reactors, Carter argued that it no longer fits in with his nuclear strategy. The Administration's plan essentially rests on the assumption that the nuclear power programme will grow much more slowly than once anticipated; uranium supplies will thus be adequate to fuel light water reactors for several decades, and breeder reactors will not be required to augment fuel supplies by producing plutonium.

The move to downgrade the breeder reactor programme was coupled with another move away from the use of plutonium in the United States. Carter also announced that the United States would defer indefinitely the reprocessing of spent reactor fuel to recover plutonium and recycle it as a reactor fuel. The announcements were designed to bolster the Administration's attempts to dissuade other countries from entering the plutonium economy, the argument being that the United States could not credibly argue against the use of plutonium without renouncing such use itself.



Camera Press

Carter: breeder setback

Although those assumptions were swiftly and sharply challenged by the nuclear industry, until recently it seemed that Carter's plan would be approved by Congress. Fierce lobbying by the nuclear industry and its supporters has, however, produced results.

The first indication that the Clinch River plant may be reprieved came last month when the House Committee on Science and Technology voted to authorise the full $150 million for the project next year, an amount which would allow construction to begin and the plant to be put back on schedule. The House has yet to act on the committee's recommendation, however.

The next move came in the Senate Commitee on Energy and Natural Resources, when supporters of the project, led by Senators Frank Church and Henry Jackson, also proposed that the Clinch River plant should be given $150 million. The committee split 8–8 on the proposal. Church and Jackson then proposed a compromise measure: $75 million should be budgeted for the plant, but construction should be deferred for a year. The rationale, essentially, was that the Carter Administration should use the year's delay to try to persuade other countries not to build their own breeder reactors; if the persuasion failed, the Clinch River project should be given the green light. The committee again split 8–8 on the proposal. Last week, however, after two days of debate, the Senate approved the compromise Church–Jackson proposal by a vote of 49 to 38.

Attention is now being focused on the House, which is expected to take up the mater next week. Though the Science and Technology Committee has proposed giving the Clinch River plant he full $150 million, a compromise proposal along the lines of the Church–Jackson amendment is expected to be offered. Lobbying on both sides is fierce, but at this stage few people are willing to predict the outcome.

Colin Norman

EUROPE

Ariane's price

IF evidence is needed that the problems of international cooperation in science are more often political than scientific, the experience of the European Space Agency (ESA) is worth considering. Right now it is in the middle of its latest lurch towards compromise.

The immediate problem is money—about 7.7 million units of account (mua) of it (1 ua=£0.622). That is the amount needed to cover the extra costs imposed by the decision to transfer the launch of the scientific satellite Exosat from a US Thor Delta rocket to ESA's own heavier Ariane launcher.

The decision was reached when ESA's Council met at the end of last month, and is frankly admitted to have been political. The original plan was for a Delta launch, and tenders for the spacecraft were submitted on that basis. But an Ariane launch was always possible, and Ariane is expected to have completed four development flights (three of them with payloads) by 1980-81, when Exosat's mission of measuring cosmic X-ray sources is due to begin.

With so much money invested in Ariane and a flight available anyway, not to use it for Exosat would, it was felt, have reflected a lack of confidence. Recent unfortunate experiences with the Delta rocket, however—most spectacularly with Geos—have apparently had little influence.

When the change came, the decision was not unanimous. Ariane being a French project, France was its strongest supporter. Britain alone voted against, both in the Council and earlier in ESA's Science Programme Committee (SPC).

ESA's science advisory committee, which unlike the SPC and Council is not an executive body with national delegates, also resisted the idea. Its membership of six scientists considered the matter at the end of last year and expressed reservations about both the delay and the risk involved in an Ariane launch.

The worries are straightforward. The scientists want the satellite reliably placed in orbit as soon as possible. Estimates of the likely hold-up vary from three months to a year; it could be longer if Ariane itself goes awry, but if that happens it's probably back to the Delta. One scientist describes any delay at all as "very serious".

Meanwhile, ESA's Council meets again next week, to decide where the extra money will come from. The SPC says it can accept only 4 mua of the total. Britain objects to using funds already allocated for science on a launcher. Delays on Aerosat may ameliorate the problem, but a launch of a second Geos, at a cost of 14 mua, must also be considered. A decision on that is due in September. The options the Council has are not unacceptably limited, and some compromise seems possible. If so, it could be a prelude to something bigger later on if and when ESA's whole programme comes up for discussion.

Chris Sherwell

USSR

Messages from without

Vera Rich describes recent developments involving Soviet dissidents, and speaks to Mark Azbel

FORTY Western nuclear physicists attending the Tenth International Accelerator Conference in Dubna last week sent a message of sympathy to Dr Yurii Orlov, the physicist and dissident who since last February has been held incommunicado in the Lefortovo prison in Moscow in connection with his membership of the unrecognised 'Helsinki monitoring group'.

The scientists' action, on behalf of a colleague whose concern for academic freedom has led him into an active role in the human rights movement, reflects a growing concern with the fate of dissidents like Orlov and a recognition that such protests can be a way of offering genuine help. In the past certain scientists have expressed doubts that this type of action might only exacerbate an already dangerous situation, but since the Twentieth Party Congress in 1956 the Soviet authorities have shown a greater sensitivity to world public opinion, especially when that opinion is voiced by scientists. As Dr Evgenii Levich explained last week at a dinner in Oxford in honour of his father, who was unable to obtain a visa to attend his own 60th birthday conference, "The Soviet authorities are unable to imagine that there is any group of people as important as scientists".

This special status, of course, cuts both ways. According to Professor Mark Azbel, who for the past 2½ years ran the illegal Sunday seminar for refusnik scientists in Moscow, it is particularly difficult for physicists wishing to emigrate to receive visas. "I think that this is related to the fact that the Soviet officials are backward", he says; "they still remember only one thing—that the physicists were the ones who invented the nuclear weapons". For that reason, apparently, mathematicians (excluding applied mathematicians) generally find it easier to get exit visas than others. Not that mathematicians have it easy: Anatolii Shcharanskii, who for some time acted as spokesman of the Seminar and was an active member of the Moscow Helsinki group, is now facing a treason charge.

Azbel was finally allowed to emigrate two weeks ago. He told *Nature*: "I am infinitely happy to become, at last, a free man and a scientist in my native country (Israel). I am infinitely unhappy, however, when I think of all my many friends who are still living in a frightful situation which I know only too well". This, he said, amounted to "gradual scientific death because of the breaking off of scientific contacts, because of lack of laboratories, because of problems with publications, because of absence of conditions for scientific work, to say nothing of the destruction of the health of both the scientists and the members of their families . . . because of the constant persecutions."

Azbel says there are still more than 100 refusnik scientists known personally to him, and that the total number "might be twice as large". This figure, of course, does not include dissidents who do not wish to emigrate. "Western scientists can do a lot," says Azbel, "as the Soviet authorities understand that collaboration with Western scientists is badly needed and that, at the same time, Western scientists do not depend on political fluctuations and that therefore they can hardly be influenced". He himself is certain that it was only due to "all those thousands of people throughout the world—both known and unknown—who supported us in our fight for scientific freedom . . ." that he was finally granted a visa. He urged that each individual case of oppression of a scientist should be taken up by his colleagues.

Ironically, the Soviet Academy of Sciences adopted a resolution last February on the "moral responsibility" of scientists to help their less fortunate colleagues. This was proposed by Corresponding-Academician Veniamin Levich, who, although a refusnik, still keeps his position in the Academy. The resolution was accepted and, as Levich says, is therefore "difficult to ignore", even though it may have no effect on his personal fate. Still, while his birthday conference was taking place in Oxford, Academician A. A. Fedorov, visiting Britain as part of a "peace" delegation, made a statement which suggested that the constant barrage of protests was beginning to take effect in Levich's case. "I hope and believe," he declared, "that Levich will be able to leave the Soviet Union very soon."

- Ernest Akselrod, a psychiatrist formerly with the space programme, who was dismissed on applying for an exit visa to Israel and has since studied psychological and medical problems of refusniks, is to face trial for "parasitism" after refusing to take an alternative job.

USSR

- The Supreme Soviet recently took time out from discussing the new constitution and assenting to Mr Brezhnev's elevation to the Presidency to consider the state of the country's forests. The current Five Year Plan provides for an extensive forestry programme, including reafforestation and planting of some 12 million ha, draining of 1.5 million ha of waterlogged plantations, and routine maintenance of some 230 million ha; the total cost is over 160 million rubles.

The main shortcomings, it appears, lie in the spheres of protection and 'integrated' use of resources. There are frequent cases of wide-spread fires as checks on the observance of fire safety rules and prevention work are not always all they should be. Too little attention is given to the use of scraps and firewood timber which is often left at the felling site. Some timber is lost during rafting and transport or disappears as waste. There is a basic imbalance in the use of timber; the conifer reserves of the Urals and the north are being consumed at an intensified rate, and insufficient use is being made of deciduous varieties.

To remedy deficits in the processing industry, another source of losses, a resolution was passed "On measures to improve further the conservation of forests and the rational use of timber resources". The usual wide campaign of support in the media soon followed. The Deputy Minister of Forests of Byelorussia suggested in an article that new measures may involve a major administrative change; those forests which still belong to collective farms (some 20% in Byelorussia), he said, should not be left "with the former owners", but handed over to the state forestry fund.

- It is only recently that the Soviet Union has acceded to the idea of international control of whale fisheries, and the statement of Vladimir Tveryanovich, chief specialist at the Ministry of Fisheries, marking the close of the whaling season, has something of the enthusiasm of the newly converted. Inverting the customary Soviet pride in overfulfilled norms, Tveryanovich proudly announced that this year the Soviet whaling flotillas had reduced the total number of whales taken; nevertheless, he added, "rational use" of the catch meant that both the variety and the quality of the output of the factory ships had been substantially improved. He also noted that scientific teams—hydrobiologists, biologists and technologists—had sailed with the flotilla; their results, he said, will allow correct estimates of whale-stocks to be made so that the whaling industry can be conducted on a more "rational" basis. There is, as always, no suggestion of a moratorium.

In the case of the dolphin, however, these is a strong Soviet commitment to a total ban on hunting, both in the Black Sea and in the Pacific. According to Aleksei Yablokov, a prominent Soviet biologist, although dolphin hunting is totally forbidden in the Soviet Union, Bulgaria and Romania, the Turks still hunt Black Sea dolphins on a large scale. He quoted "figures from foreign experts" indicating that at least 40,000 dolphins are landed from the Black Sea, and almost as many escape from the hunters only to die of wounds. Several Soviet surveys of the Black Sea dolphin population have been made during the past few years; with the present rate of catch, says Yablokov, the Black Sea dolphin will be virtually extinct by the end of the century. As for the Pacific dolphin, between hunters and pollution it may well become a disappearing species within "a couple of years" unless some action is taken.

Yablokov stressed that dolphins are not a suitable source of raw material for industry. The skins and fat are of low value, and their meat is inedible. This last statement contrasts curiously with the campaign of 1930 which claimed that dolphin meat (in the form of sausages and other delicatessen) could make a significant contribution to the Soviet food supply, and even earn foreign currency as an exotic export. According to Yablokov, the dolphins still have a role to play in the Soviet economy. Experiments are going forward on the training and domestication of Tursiops dolphins, which tolerate life in captivity well and will breed in captivity.

Whether these somewhat exotic plans for the dolphin materialise, Yablokov's other domestication project seems more feasible. This concerns a small school of baleen whales, living near the Shantar Islands. Since the baleen whale has a relatively small range, Yablokov suggests that they might be herded in a similar manner to reindeer, with herdsmen seeking out the best "pastures" for them, and protecting them from their natural predator, the killer whale.

- The discovery of a baby mammoth, preserved in the permafrost, is proving a most exciting challenge to Soviet palaeontologists. It was found by a bulldozer operator cutting peat on the banks of the Kirgili river in the Magadan goldfields. According to Academician N. A. Shilo, the head of a special commission investigating the find, it is the best-preserved specimen so far discovered. Interviewed in *Pravda*, he said the young mammoth was 115 cm long, 104 cm high, and had a trunk length of 57 cm. The body was covered with reddish-brown hair. It was, apparently, some six months old at the time of death.

- A new 'primary standard' based on a special time and frequency system came into force throughout the Soviet Union on 1 July. The latest time standard, designed at the Institute of Radio Physics and Electronics of the Academy of Sciences of the Ukranian SSR, is based on a ruby rod maintained at 2 K which transforms electromagnetic energy into acoustic waves with a frequency of 10^{10} Hz.

At present the Soviet Union has 105 State Standards; by 1980 there should be some 140. According to V. I. Kipichenko, Head of the Metrology Board of Gosstandard (State Standard Committee), the fundamental standard of length—defined in terms of the wavelength of ^{86}Kr radiation—"is not proving fully satisfactory in present-day conditions". An intensive research programme is accordingly under way to produce a standard of length based on laser radiation which, he says, will make it possible to link the standard length to that of time.

Vera Rich

correspondence

The laetrile question

SIR,—There is no lack of "freedom to use harmless drugs", but freedom to promote and sell worthless remedies is constrained. Laetrile (which is not harmless), is the latest and most ingeniously promoted of a long string of such money makers. Croft's first consideration (14 July, page 100) implies that because scientists have been wrong before, therefore they are wrong this time. It is a favourite ploy of laetrile promoters' modesty to compare themselves with the Wright brothers, Louis Pasteur and other shatterers of precedent.

His second consideration, the "resentment of the wealth that medical doctors extract" is an example of the "principle of compensatory evil". Apparently it is alright for doctors to make money by prescribing laetrile. His third point seems to imply that the consumption of a cyanogenic glycoside is part of the pursuit of happiness. Unfortunately, the quest sometimes ends with the use of a stomach pump.

Would Jukes turn people away from Lourdes? Alas, I might if the admission fee were $1,500, coupled with a promise to cure cancer.

Yours faithfully,
THOMAS H. JUKES

Nutrition planning

SIR,—In his attack on the present state of nutrition planning (30 June, page 742), Donald McLaren makes some characteristically trenchant points regarding the nature of the world's food 'problem', but seems to be attacking the wrong target. Surely the failure of the Green Revolution, as failure it probably is, is a classic case of the non-holistic, piecemeal approach which he advocates. It was precisely because (for reasons not unconnected with that inelegant term, agripower) no thought was given to the economic, educational and organisational needs associated with introduction of improved crop varieties, that the increased potential yields of these varieties did not manifest themselves as improved nutrition for the mass of poor consumers.

To implement lasting improvements in nutrition, it is surely necessary to investigate not only biochemistry, but also land tenure, demography, the availability of water and fertilisers, political power-mongering and so on, for the relevant area. Such an analysis is, by definition, holistic. As McLaren himself points out "the widespread problem (. . . .) has socio-economic roots".

I share McLaren's doubts regarding "Utopian social engineering" and "holistic day-dreaming"; similarly the systems engineering approach of the space programme is likely to be totally inappropriate in tackling nutritional problems. Despite this, a broader approach to the study of nutrition, involving economic and institutional factors, even, possibly, the occasional maze of flow diagrams, seems to be essential. This is certainly the approach adopted in our 1978 Open University course, Food Production Systems, of which McLaren's paper on 'The great protein fiasco' (*Lancet* 93–6 1974), forms an integral part.

Yours faithfully,
R. M. MORRIS
Open University, Milton Keynes, UK

Unemployed scientists

SIR,—I agree with Dr Klein's comments (23 June, page 665) and would like to raise a related issue. The dire problem of post-PhD employment is still to be fully debated in Britain. The number of qualified scientists in their late twenties and early thirties with no permanent job is rising fast, especially in biology. Universities seem to be passing such people over when filling posts recently, and the reasons for this are twofold: young (ie early twenties) post doctoral job applicants are cheaper to employ and, perhaps more insidious, there is a feeling that older candidates may compete for seniority with their longer employed, colleagues of equal age.

In the long term, such practices must be harmful to the academic community. In few other countries can an individual of 23 or 24 years of age, even perhaps five years older, be regarded as intellectually mature enough to acquire a permanent post tenable for the remainder of his working life. Here in Germany for example, the normal procedure is to spend up to six years as an *Assistent* before competing for a permanent university post.

I would like to see a list of senior, non-tenured scientists drawn up for each discipline, and circulated to the relevant university departments. Any PhD would be eligible to join this list at age thirty or so. Before advertising any post, the university concerned should first consider senior scientists on the list, and should offer the job to any suitable candidate thereby found. If none are eligible, then the post could be freely advertised.

By using a system of this kind, the backlog of skilled labour would rapidly be incorporated. Post doctoral research funds would take care of younger people waiting their turn, and an accompanying drastic decrease in PhD student numbers for the next few years would help shorten the queue.

Yours faithfully,
ROBERT RANSOM
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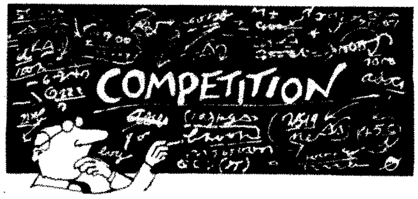


Competition 14. £10 for the most stultifying, dreary or meaningless paragraph or two on any theme or non-theme (either published or of your own invention). The second leader of *Nature* **258**, page 465 (11 December, 1975) may serve as an example. Closing date: 26 August.

Competition 13 asked for a technological message to a less advanced civilisation out in space. Jeanne Hopkins, on the staff of the *Astrophysical Journal* in Chicago, was a clear winner with the following:

'Congratulations on inventing the wheel! Yes, we do realise that all the tedious manual labour was performed by your grad students—but the idea was yours, and that's what counts. As you have no doubt informed your Grants Committee, your invention will revolutionise warfare; however, massive Government funding will be required to bring it to its full capability. We suggest that you submit a detailed proposal to your government, outlining possible modifications and improvements, along with a target date for each. For example: "Round wheel, target date 30 years. Toothed wheel, 300 years. White sidewalls, 3,000 years." '

news and views

Approaches to effective anti-cancer drugs

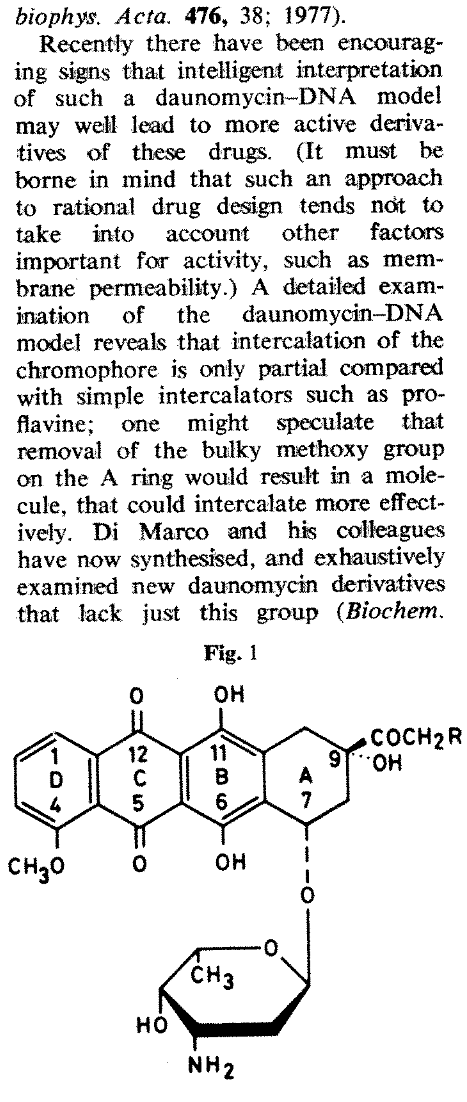
from Stephen Neidle

ONE of the most sobering guiding principles in the development of new drugs is that it is often exceedingly difficult to improve on what nature has already provided. This tenet is especially true in the case of the topical anti-cancer agents daunomycin (R=H) and adriamycin (R=OH) (Fig. 1), first isolated some years ago from cultures of *Streptomyces peucetius* by Di Marco and his associates at the National Cancer Institute, Italy. These two compounds do indeed have considerable clinical usefulness, particularly in the treatment of acute leukaemia; adriamycin has recently achieved promising results with solid tumours as well. However, apart from being cytotoxic, (as are all anti-cancer drugs), they suffer from the very serious drawback of having severe cumulative dose-dependent cardiotoxicity (Lenaz & Page *Cancer Treatment Rev.* **3**, 111; 1976). Much effort has been directed to obtaining either new derivatives, or to developing new dose regimes, that show decreases in undesirable side effects and/or increased anti-cancer activity and selectivity. Such goals are common to many areas of cancer chemotherapy; however, in the case of daunomycin and adriamycin, it is now possible, at least in principle, to take account of recent knowledge concerning their mode of action.

It is now generally accepted that these drugs have as their primary cellular target, nuclear double-stranded DNA, to which they most probably bind by an intercalative process. The consequent inhibition of both DNA and RNA synthesis is more pronounced for cancerous cells, with their higher turnover rate. Some years ago, Pigram, Fuller and Hamilton suggested a generally plausible model for the interaction of daunomycin with DNA, based on X-ray fibre diffraction data and molecular model-building. They suggested that the aromatic chromophore of the drug is intercalated in between adjacent partially unwound base pairs, and the sugar is hydrogen-bonded to the nucleic acid backbone through its amino group. The model satisfactorily explains several features of drug–DNA binding behaviour, perhaps the most striking being a very marked diminution of both binding and biological activity when the sugar stereochemistry is altered, by, say, configurational inversion (Di Marco *et al.* *Cancer Res.* **36**, 1962; 1976; *Biochim. biophys. Acta.* **476**, 38; 1977).

Recently there have been encouraging signs that intelligent interpretation of such a daunomycin–DNA model may well lead to more active derivatives of these drugs. (It must be borne in mind that such an approach to rational drug design tends not to take into account other factors important for activity, such as membrane permeability.) A detailed examination of the daunomycin–DNA model reveals that intercalation of the chromophore is only partial compared with simple intercalators such as proflavine; one might speculate that removal of the bulky methoxy group on the A ring would result in a molecule, that could intercalate more effectively. Di Marco and his colleagues have now synthesised, and exhaustively examined new daunomycin derivatives that lack just this group (*Biochem. biophys. Res. Commun.* **69**, 744; 1976). The modified molecule does indeed bind to DNA somewhat better than its parent. Significantly, *in vivo* testing of these 4-demethoxy derivatives in mouse cancer systems, both of the leukaemic and solid tumour type, show that it is as effective as daunomycin itself, but at dose levels four to eight times lower (Di Marco *et al.* *Cancer Treatment Repts* **60**, 829; 1976).

Fig. 1



The notion of targeting drug molecules specifically to aberrant cells, thus increasing their effectiveness, is a relatively new one. Many types of carrier for the drug can be envisaged ranging from specific antibodies to whole cells. Perhaps the classical example is the DNA–daunomycin complex proposed by Trouet (*Nature new biol.* **239**, 110; 1972) which has, perhaps unsurprisingly, proved to be of questionable value in a clinical context. However, recent reports of the successful use of liposomes (phospholipid vesicles) as versatile general carriers for, amongst others, a DNA–daunomycin complex, suggest that some of the earlier problems, such as susceptibility to endonucleases, can be overcome (Gregoriadis *Nature,* **265**, 407; 1977). Another quite distinct approach has been developed by *Varga et al.* (*Nature*, **267**, 56; 1977). They have capitalised on their earlier findings that the peptide melanotropin binds specifically to surface receptors of mouse melanoma cells; a daunomycin–melanotropin complex was also found to bind strongly to the cells *in vitro,* with a threefold molar increase in toxicity compared with the free drug. Moreover, the complex was not toxic to non-malignant mouse fibroblasts. One awaits with interest the extension of this promising approach to other cell lines, and *in vivo* systems.

The carrier concept in its various manifestations has the potential of, at least in part, overcoming the serious cardiotoxicity of daunomycin and adriamycin. The origins of this property, unique (fortunately!) to these

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drugs remain largely unknown, certainly at the molecular level, in spite of much speculation. A suggestion by Folkers (*Proc. natn. Acad. Sci. U.S.A.* **73,** 4653; 1976) that inhibition of mitochondrial coenzyme enzymes might be an important factor, has been taken up by Lown (*Biochem. biophys. Res. Commun.* **76,** 705; 1977), who suggests modifications to the anthraquinone chromophore of the drugs as a means of surmounting the cardiotoxicity. However, it does seem that such alterations would result in the undesirable effect of markedly decreasing DNA binding ability since the basic requirement for intercalation, a planar chromophore, would no longer be so well obeyed. This illustrates one of the pitfalls of this approach to molecular drug design—how optimisation of one characteristic often results in deleterious effects to others. ☐

Elephants in Uganda

from Robert M. May

For the elephants of Uganda, life has got worse under General Amin. This is supported by the trends recently reported for the number of elephants in Kabalega Falls (formerly Murchison Falls) and in Rwenzori (formerly Queen Elizabeth) National Parks (Eltringham & Malpas *Oryx* **13,** 334; 1976; Eltringham *E. Afr. Wildl. J.* **15'** 19; 1977 and personal communication). The figures in the table are average numbers of elephants for wet seasons only, and are based on aerial sample counts (Eltringham *E. Afr. Wildl. J.* **10,** 299; 1972). The asterisks on the numbers for 1976 denote that they are uncorrected total counts, and therefore overestimates.

These and other African Parks were created at a time when the amount of habitat available to large animals was severely decreasing due to increases in the human population. Until the late 1920s, elephants ranged over 70% of the land area of Uganda; by 1960 the figure was 17%. As a result, large animals have tended to crowd into game reserves and National Parks, often at densities above the carrying capacity of the environment. The problem is particularly acute for elephants, whose feeding habits of uprooting and debarking trees can be very destructive. By the mid-1960s, conditions in the Murchison Falls National Park had so deteriorated that it was feared the Park would erode into a near desert, and 2,000 elephants were shot in an attempt to alleviate matters (Laws, Parker & Johnstone *E. Afr. Wildl. J.* **8,** 163; 1970). The problem of environmental degradation wrought by elephants has arisen in other East African National Parks, most notably Tsavo, and opinion is divided both about the ultimate cause and about the cure (Caughley & Goddard *E. Afr. Wildl. J.* **13,** 39; 1975; Laws *Oikos* **21,** 1; 1970). Neither the optimal density nor the optimal age structure are well understood.

Into this vexed situation, the numbers tabulated above introduce a grim note. The precipitous decline that in 3 years has reduced the elephant population in Rwenzori by a factor of four, and in Kabalega Falls by a factor of ten, is clearly due to poaching for ivory. No attempts have been made to use the carcasses for food, nor to remove the valuable skin from the ears (an elephant hide attache case sells for $600 to those crass enough to carry one). The elephant populations have not even enjoyed the benefits of a thinning, but rather the distribution has changed, with the animals now concentrated into relatively few pockets, where, presumably, they are safest. Eltringham and Malpas observe, moreover, that "the indiscriminate slaughter has caused a breakdown in the normally well ordered family structure: indications of a collapse in the social order are to be seen in the clumping of family units into large amorphous herds. Normally, the family is led by the matriarch which, being the oldest in the group, has large tusks and is, therefore, the poacher's prime target. With the loss of the matriarchs, the leaderless elephants have tended to congregate together, so that locally their density remains high and damage to the vegetation continues."

The experience of the past 20 years has shown that, even with the best will in the world, there are formidable ecological problems in the management of wildlife preserves. For the East African National Parks, the income generated by tourism has provided a practical motive for grappling with these management problems; with the decay of Uganda's tourist industry, it seems that mere anarchy is loosed upon the elephants.

Time	Kabalega Falls (Southern sector)	Rwenzori
Average of earlier counts	8,042	2,633
September, 1973	10,187	2,864
September, 1974	4,072	1,868
September, 1975	1,061	931
September, 1976	1,346*	704*

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Binding the *lac* repressor

from Alan D. B. Malcolm

The *lac* repressor protein binding to *lac* operator DNA is the classic example of specific protein–DNA binding, and as such is the best characterised in terms of molecular structure. But it is still not clear why the repressor binds that particular DNA sequence so tightly and how interaction of the repressor protein with inducers such as isopropyl thiogalactoside (IPTG) completely abolishes its capacity to bind operator.

The repressor consists of four identical subunits whose primary sequence is known and studies of repressor mutants have shown that it is the N terminus which is involved in operator binding. Recently (*J. biol. Chem.* **251,** 3386; 1976) Files and Weber showed that trypsin treatment of the repressor removes the N terminal 59 amino acids from each chain leaving a tetrameric core which still binds inducer but does not bind DNA. Geisler and Weber (*Biochemistry* **16,** 938; 1977) have now found conditions for the tryptic digestion so that the intact 1–59 peptide may be isolated (albeit in a mixture with the 1–51 peptide as well). The 1–59 peptide has considerable secondary structure and can bind DNA. However it does so only weakly—with about the same dissociation constant with which the intact repressor binds non-operator DNA. This binding is still thought to be biologically significant however, because kinetic studies suggest that the repressor first binds to any (non-operator) sequence and then moves along the double helix until it locates the operator where it then binds 10^8 times more tightly.

The original operator region sequenced by Gilbert and Maxam consisted of the 27 nucleotides protected by the repressor from enzyme digestion. When the sequence was subsequently extended to 35 base pairs it was clear that there were four regions related by

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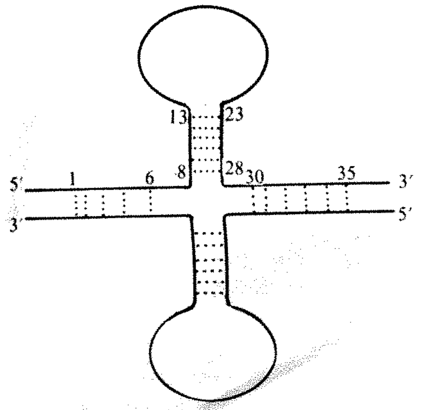

a twofold axis of symmetry—base pairs 1–6 are rotationally related to base pairs 30–35, and 8–13 and 23–28 are similarly related. This of course allows the possibility that the double helix be looped out to form cruciform structures as suggested over ten years ago by Gierer. Jones and Olson (*J. theor. Biol.* **64,** 323; 1977) have now suggested that if the N terminus of the protein is folded into a π helix (which has five amino acid residues per turn), one side of the helix will have tyrosines 7, 12 and 17 sandwiched between Lys 2 and Arg 22 (model studies in many laboratories have shown that such peptides interact strongly with nucleic acids) whereas the opposite face is fairly hydrophobic and would be ideal for facing in to the core of the repressor. If the operator sequence has one looped out section as shown then Jones and Olson are able to propose a perfect fit between the four subunits of the repressor and the four hydrogen bonded regions of the operator.

Unfortuntely this attractive hypothesis has to face several ugly facts. The first is that a search for single-stranded regions in the operator using specific nucleases has failed to reveal any (Marians & Wu *Nature* **260,** 360; 1976). Such looped-out sequences are also out of favour in the λ operator where an expected effect on superhelical density has not been observed. Even more significantly Narang and his coworkers have been synthesising various sections of the operator and testing them for repressor binding. They showed last year (*Proc. natn. Acad. Sci. U.S.A.* **73,** 91; 1976) that the 21-nucleotide long fragment from position 8 to 28 inclusive retains full binding activity although it does not contain either of the outer palindromic sequences. Even more surprisingly, they have now shown (*Proc. natn. Acad. Sci. U.S.A.* **74,** 966; 1977) that the 17-nucleotide long region from 10 to 26 still binds although two base pairs from each of the inner palindromic sequences are now also absent. It is something of a relief to find that anything smaller than this heptadecanucleotide does lose repressor binding ability. It may be that many of the palindromic sequences so far found at operator and promoter sites in prokaryotes are not significant after all, especially in view of the finding (Valenzuela *et al. Nature* **267,** 641; 1977; Maxam *et al. Nature,* 643; 1977) that the 5S RNA promoter in yeast has no palindromic regions at all.

Chemical syntheses of the operator region in which 5-bromouridine or deoxyuridine have been substituted for thymidine have been reported (Yansura *et al. Nucleic Acids Res.* **4,** 723; 1977) and all the various 'mutants' tested bind repressor as well as does the wild type. It looks as though the *lac* operator is going to stretch the abilities of synthetic chemists and biophysicists to the limit before it yields the secret of its repressor binding. ☐

Nitrate in drinking water: health hazard unlikely

from Alan Wild

NITRATE has variously been blamed for algal blooms in lakes and rivers, for methaemoglobinaemia in young babies and most recently as a possible cause of gastric cancer. Fears have been expressed that increased use of nitrogen fertilisers on arable land will lead to a higher nitrate concentration in the water supply, and the Royal Commission on Environmental Pollution in the UK is at present considering the impact of agricultural practices on nitrate concentration in the water supply.

In the UK the Water Authorities follow the WHO standards laid down in 1970 which, for drinking water quality in general, are regarded as 'over-safe' for the UK (Pittwell *J. Institution of Water Engineers and Scientists* **31,** 214, 1977). The WHO recommended concentration of NO_3–N per litre is less than 11.3 mg, 11.3–22.6 mg l^{-1} is "acceptable" and more than 22.6 mg l^{-1} is unacceptable. These standards are based on work in 1945 in which well water contaminated with coliforms (indicators of faecal pollution, another source of high nitrate levels) containing 90 and 140 mg l^{-1} of NO_3–N was implicated in an illness of two infants which was diagnosed as methaemoglobinaemia (Comly *J. Am. med. Assoc.* **129,** 112; 1945). Comly recommended that well water used for infant feeding should possess a NO_3–N content no higher than 10 or at most 20 mg l^{-1}. In the US and several other countries threshold standards of 10 and 20 mg l^{-1} are used. It may be noted that the WHO standards are derived from 50 and 100 mg NO_3 l^{-1}, and 11.3 and 22.6 may imply an accuracy that was not intended.

More recently the fear that nitrate may be implicated in gastric cancer arose from speculations that amines and nitrite react in the body to form carcinogenic nitrosamines, and that the nitrite is formed by reduction of nitrate. There is only circumstantial evidence for this mode of formation of carcinogens (see Mirvish *Specialised Conference on Nitrogen as a Water Pollutant,* Copenhagen; 1975; Doll *Nature* **265,** 589; 1977), and it must be considered yet unproven. It should be noted that with methaemoglobinaemia and nitrosamine formation the nitrate (and nitrite) may come from food or water.

As a society we have to balance the health risk of nitrate in water (and food) against the cost of any requirement to reduce the concentration. The risk is extremely small: only one death in the United Kingdom was attributed to nitrate in water in the period 1950–75, and this followed the use of well water polluted with coliforms (Tayler *Water Treatment and Examination* **24,** 194; 1975). No cases were reported during the drought of 1976 (*House of Commons Parliamentary Debates* 22 February, 1977).

Nitrate concentrations do not often exceed 10 mg l^{-1} in public water supplies, but if they rise above the recommended standard of 11.3 mg l^{-1} the Health Authorities are automatically notified and may issue warnings to parents of young infants to use bottled water.

Concentration in river water in UK

Concentrations in river water become highest during the winter months; this is attributed to drainage from agricultural land (Slack *J. Institution of Water Engineers and Scientists* **31,** 43; 1977), and Water Authorities may then need to blend waters or withdraw some sources from use. Although analyses for 1976 have not been reported in full, it appears that there was an unusually large rise in concentration after the dry summer; for example in the two rivers Avon (Bristol) and Stour, concentrations rose to more than 11.3 mg l^{-1} in October 1976 and were over 22.6 mg l^{-1} for a short period

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(Toms *Seminar Institution of Water Engineers and Scientists* 29 March, 1977).

It is obviously important to know what nitrate concentrations in rivers and aquifers we can expect in the future. Extrapolating from a survey of nitrate in 18 rivers in England and Wales during 1953–67 (Tomlinson *Water Treatment Examination* **19,** 277; 1970) gradual increases can be expected in some rivers but not in others. This is borne out by an increase in the annual average from 7.1–8.8 mg l^{-1} in the river Trent and a small drop in the Severn from 3.8–3.7 mg l^{-1} between 1971/73 and 1975/76 (data abstracted from *Water Quality* 1975/76, Severn Trent Water Authority). The response of aquifers to nitrate input occurs more slowly, but concentrations as high as 40 mg l^{-1} have been found in the unsaturated zone above the Chalk aquifer in Hampshire (Young *et al. Water Research Centre Tech. Rpt* TR 31; 1976). In accordance with results from elsewhere, concentrations were related to ploughing of old grassland and to fertiliser use, especially the former. From the known dates of these events it was calculated that the groundwater moves through the unsaturated zone at a rate of about 1 m per year, in agreement with estimates for tritium migration, and thus higher concentrations of nitrate may reach the aquifer in 20 to 30 years' time. It is not yet known whether, before reaching the aquifer, concentrations will fall to safe levels by denitrification or by mixing with low-nitrate water.

Relative contribution from various sources

There seems to be no estimate for the United Kingdom of the relative contribution of urban, industrial and agricultural sources to nitrate in river water but an estimate from the Netherlands is that 40% of the nitrate entering watercourses is from urban sewage effluents (Cooke *MAFF Tech. Bull.* No. 32, 5; HMSO, London, 1976). Again in the Netherlands, it has been estimated that of the total 32 kg $\mathrm{NO_3}$–N ha^{-1} entering the watercourses from agricultural land, 20% comes from fertilisers, 15% from organic manures and 65% by mineralisation of soil organic matter (Cooke *MAFF Tech. Bull.* No. 32, 5; 1976). There is thus evidence here, and from the work of Young *et al.* that ploughing of old grassland is a major agricultural source of nitrate. In the UK the area (million hectares) under permanent grass decreased from an average of 7.6 in the 1930s to 4.9 in 1946, increased to 5.5 in 1955–57 and declined gradually to 4.9 in 1973 (*Agricultural Statistics for the United Kingdom,* HMSO, London), indicating that the practice of ploughing old grassland has become uncommon. Unfortunately there is little information on the loss of nitrate from sown grassland which is a few years old, or from improved hill grazing land.

Under well manured arable crops the loss from soil and fertiliser nitrogen has been shown to give concentrations of nitrate in the land drains averaging 10 to 18 mg l^{-1} at Rothamsted, Woburn, Saxmundham and Broom's Barn Experimental Stations (Williams *MAFF Tech. Bull.* No. 32, 174, 1976) with higher concentrations in the autumn and after a wet spring. Under heavily manured grassland the concentration in the drainage water is usually less than 10 mg l^{-1} (Hood *MAFF Tech. Bull.* No. 32 201; 1976) and in drainage from unfertilised grassland the nitrate concentration is often less than in the rainfall (about 1 mg l^{-1}) (Cooke *MAFF Tech. Bull.* No. 32, 5; 1976). The most serious problem is therefore under arable crops, which occupy about half the farmland in England and Wales. It was estimated in 1973 that cereals, accounting for three quarters of the arable area received about 10% less nitrogen than that recommended for optimum yields (Church *MAFF Tech. Bull.* No. 32, 92; 1976). A fair conclusion seems to be that the output of nitrate into surface and ground waters from agricultural sources will increase only a little in the future although there will be differences between crop seasons determined by the weather. Nevertheless, when taken together with increased output of nitrate from sewage treatment to reduce the biological oxygen demand of the effluent, and recycling to meet an increased demand for water, the trend of nitrate concentrations seems likely to be upwards, exceeding 11.3 mg l^{-1} more commonly in the future.

The threshold limits set for safe concentrations of nitrate in drinking water are now seen to be critical. There is no evidence to support the WHO limits and we should recognise the absence of risk to adults with concentrations of 22.6 mg l^{-1}, a requirement that Water Authorities can easily meet. With young infants it seems advisable to keep to an upper limit of 11.3 mg l^{-1} until evidence indicates otherwise, and to supply low-nitrate water in bottles as required. An 'oversafe' limit will increase the costs of purifying drinking water and of sewage treatment quite unnecessarily, and might bring unreasonable pressure on farmers to restrict the use of fertilisers (which now produce food worth about £1,000 million per annum). At the same time there is a clear requirement to establish from sound medical evidence the upper permissible limit especially for young infants and to improve the models used for forecasting changes in nitrate concentrations, which means identifying sources and measuring rates of denitrification in surface and ground waters. It is to be hoped that the Royal Commission on Environmental Pollution will allay the unjustified public anxiety over nitrate in drinking water and also point to ways of meeting these two requirements. ☐

Insulin genes into *E. coli:* first step from fantasy to fact

from Peter W. J. Rigby

ONE of the major benefits to accrue to society as a whole from the exploitation of *in vitro* DNA recombination may well be the production by industrial fermentation of medically important proteins such as antibodies and hormones, which are expensive to isolate from animal sources. The most popular scenario of this type involves the production of polypeptide hormones, particularly insulin, by isolating the relevant gene as a recombinant plasmid and forcing the expression of the inserted eukaryotic DNA within *E. coli.* A recent paper (Ullrich *et al. Science* **196,** 1313; 1977) describes the first step towards this objective, the isolation of part of the DNA sequence coding for rat insulin.

This work, performed in the laboratories of William Rutter and Howard Goodman at the University of California Medical School in San Francisco, depends on the already classical approach of copying mRNA into double-stranded cDNA using reverse transcriptase and then inserting the cDNA copy into a bacterial plasmid vector. So far most work of this type has involved genes such as globin, whose mRNA can be obtained in highly purified form (Efstratiadis *et al. Cell* **7,** 279; 1976). After introducing the recombinant plasmids into *E. coli* by transformation, bacterial colonies containing the desired sequence can be identified by hybridisation with an appropriate probe, generally the purified mRNA. Pure insulin mRNA is not available however, and so an indirect approach has had to be adopted. The success of this work has also depended on several ingenious technical developments which should find widespread application in recombinant DNA research.

The first problem is simply the isolation of insulin mRNA, synthesised

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only in the islets of Langerhans in the pancreas, which is a very rich source of RNase. To circumvent this problem Ullrich *et al.* used a previously described procedure to isolate islets, thus increasing the proportion of insulin-synthesising cells, and then isolated RNA by a method, developed in Rutter's laboratory, for preparing intact mRNA from sources rich in RNase.

cDNA prepared from total polyadenylated mRNA isolated in this way was characterised by gel electrophoresis both before and after digestion with the restriction endonuclease *Hae*III. Undigested cDNA showed a prominent band about 450 nucleotides in length, while the *Hae*III digest showed bands of 180 and 80 nucleotides. The strategy of the subsequent work is completely predicated on the assumption that these prominent bands are derived from insulin cDNA, which should be a major component of the total cDNA.

Double-stranded cDNA synthesised in this way is in fact a single DNA strand with a hairpin-loop structure. In the conventional strategy for cloning such cDNA the next step would be to open the loop by digestion with S1 nuclease, add homopolymeric tails and then join the tailed cDNA to a vector carrying complementary homopolymer tails. A major disadvantage of this approach is the absence of a reliable method to excise the complete insert from the recombinant plasmid. Ullrich *et al.* have overcome this problem by using chemically synthesised restriction endonuclease recognition sites as linkers. The cDNA was digested with S1 nuclease to open the loop and render it perfectly double-stranded; this blunt-ended DNA molecule was then joined at both ends to a decanucleotide containing the *Hin*dIII recognition sequence. Such blunt-ended joining can be performed using the DNA ligase induced by phage T4, rather than the *E. coli* enzyme which requires cohesive termini. The *Hae*III endonuclease fragments were similarly joined to the linker; in this case S1 digestion was unnecessary as *Hae*III produces blunt ends. Digestion of the products of these reactions with *Hin*dIII endonuclease produces DNA segments with cohesive ends, which can be joined to *Hin*dIII digested vector DNA, in this case the plasmid pMB9.

While such cohesive end joining is convenient and simple, it suffers from a major disadvantage in that the vector can recircularise and thus many of the bacterial colonies selected for expression of the marker function carried by the vector are found to contain merely the reconstituted vector, with no insert of foreign DNA. By treating the *Hin*dIII digested pMB9 DNA with alkaline phosphate to remove 5′-phosphate groups Ullrich *et al.*, prevented this recircularisation. Only those plasmid molecules which have annealed to foreign DNA carrying 5′-phosphates can be convalently closed by DNA ligase and thus the background in the transformation step is drastically reduced.

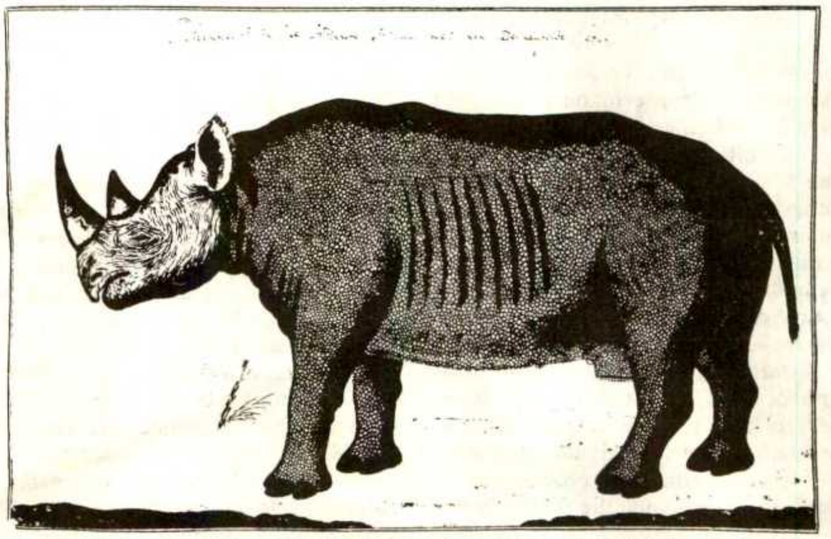

PLATE II. Gordon's second *Diceros bicornis* figure, the head more naturally poised. (Gordon Atlas, fig. 206, Rijksmuseum, Amsterdam).

Illustration from 'Robert Jacob Gordon's original account of the African Black Rhinoceros' (Cave & Rookmaaker *J. Zool., Lond.* **182,** 137; 1977). This provides an account of the memoranda and drawings comprising a description of the African Black Rhinoceros prepared at the Cape of Good Hope in 1778 by Robert Gordon and now incorporated in the Gordon Atlas preserved in the Rijksmuseum, Amsterdam. Although 'Gordon's rhinoceros' was temporarily recognised, Gordon's material never attained independent publication and the historical and anatomical merit of this pioneer investigation went largely unrecognised.

Having introduced these recombinants into *E. coli* by transformation and selecting for tetracycline resistance, the marker function carried by pMB9, the next step in the experiment would normally be to screen for the desired sequence by hybridisation. As no suitable probe was available, crude DNA preparations were made from each clone and these were screened by restriction endonuclease digestion, to look for plasmids containing inserts of 450, 180 or 80 nucleotides, the sizes predicted from the characterisation of the cDNA. One clone derived from total cDNA and containing an insert of 410 nucleotides was identified in this way. By a slight variation on this procedure clones containing the 180 and 80 nucleotide *Hae*III fragments were also obtained.

Even at this stage of the experiment there was no direct evidence that the inserts corresponded to insulin-coding sequences.

The identity of the cloned cDNA was, however, established in the most convincing way possible by direct sequence analysis which showed that it coded for the known amino acid sequence of rat insulin. The immediate product of the translation of insulin mRNA is preproinsulin, which is processed to the mature hormone by a series of proteolytic cleavages. The four plasmids isolated together cover the whole of the proinsulin coding sequence as well as part of the N-terminal pre-peptide. Unfortunately none of the clones contains sequences from the 5′ untranslated region of the mRNA.

This work, demonstrating that particular nucleic acid sequences can be isolated in the absence of any screening procedure other than direct sequencing of the final recombinants, represents something of a triumph for 'brute-force' molecular biology. However, the general applicability of such methods is unclear. Not until the sequencing was quite advanced could Ullrich *et al.* have been confident that islet mRNA did not contain a major component which was not insulin mRNA. It seems likely that if this approach were used in other cases the wrong sequence would be cloned. In this regard it is particularly unfortunate that the paper contains no data bearing on the efficiency of the procedure; it would be of great interest to know how many clones were screened to obtain the four containing insulin sequences.

We can confidently expect these plasmids to be used as probes in experiments to isolate the complete insulin gene together with its flanking sequences from total rat DNA. Once such recombinants are available it will be possible to explore approaches to forcing the expression of the insulin gene within *E. coli.* Techniques already exist to force transcription of inserted DNA, but whether such transcripts can be faithfully translated is unknown. Even if *E. coli* could be persuaded to synthesise preproinsulin there still remains the problem of processing this to the mature hormone. The use of this technology for the large scale production of insulin may well depend not only on engineering *E. coli* to express the insulin gene but on the development of *in vitro* processing systems. There clearly remains a great deal of work before one can confidently anticipate the commercial production of insulin in *E. coli,* but the first step has been taken. ☐

Evidence for Earth expansion?

from Peter J. Smith

The concept of an expanding Earth has been in circulation for several decades now, but has never attracted much interest. If it were not for the historical example of continental drift, it would be tempting to assume that such indifference reflects a correct interpretation of the Earth's behaviour and that expansion is only an illusion in the minds of a handful of enthusiasts. But the temptation should be resisted, at least for the time being. Drift was once regarded as illusory by the majority of Earth scientists; but the idea was kept alive by a few fanatics until proof came at last from outside the field of geology. Geologists, convinced that continental drift was inconsistent with the existing body of geological knowledge, suddenly found their rejected theory confirmed by physicists and were forced to reinterpret their data accordingly.

Of course, it would be dangerous to over-react to history by concluding that the majority must now be wrong about Earth expansion as it would be to reenact the sort of response which greeted Wegener's suggestion that the continents had drifted. The cases are not precisely analogous. There were serious problems with the pre-drift world view that only drift could resolve, whereas Earth expansion appears to offer no comparable advantages. On the other hand, if proof of expansion is to come at all it is most likely to come, as with drift, from the physicists. Earth scientists may feel they have no need for expansion; but if physicists could show that the gravitational constant has decreased with time, expansion would have to be accepted and accomodated.

In the meantime, however, there are still occasional attempts to test the expansion hypothesis against purely geological–geophysical data, the latest of which comes from Steiner (*Geology* **5**, 313; 1977). Steiner's idea is simple in principle. For an Earth of constant radius, the rate at which the surface area of ocean floor increases at oceanic ridges must equal the rate at which it decreases through subduction. But if the former is greater than the latter the total area of the ocean floor must *ipso facto,* increase; and the most obvious explanation of such an increase would be an expanding Earth.

Unfortunately, as previous attempts have shown, the practice of testing for expansion is invariably much more difficult than the principle. Steiner begins well enough with the map of ocean basin ages produced by Pitman *et al.* (*Geol. Soc. Amer. Map MC-6*; 1974), from which he measures directly the area of floor produced in the three major oceans during each geological epoch and period from the Jurassic to the Holocene. The first conclusion to emerge clearly is that at most times the rates of floor production vary considerably from ocean to ocean but with "a tendency toward negative correlations where a maximal rate of sea-floor spreading in one ocean basin is compensated for by a minimal rate in another". During the Paleocene, for example, the rates in the Indian and North Atlantic Oceans were high compared with those in the Pacific and South Atlantic Oceans; during the Eocene the rate in the Indian Ocean was higher than those in the Pacific and North Atlantic Oceans which were in turn higher than that in the South Atlantic Ocean; and so on.

The existence of such negative correlation indicates that sea floor spreading results from a globally coordinated process, although this no more rules out the possibility of traditional convection than it does the possibility of expansion. Slightly more favourable to expansion (though again not definitive), perhaps, is the fact that the combined rate of ocean floor production for all the oceans together has apparently increased exponentially with time since the Jurassic. Of course, this detected increase could be spurious. The ocean floor data currently available would indicate an increase even if the rate of floor production had always been constant, for the older the sea floor the greater generally is the proportion already subducted. Moreover, the picture is confused by the fact that the map produced by Pitman and his colleagues is incomplete, specifying ages for only about 66% of the deep ocean floor. To determine the true rate of floor production since the Jurassic, Steiner has therefore had to make estimates of the spreading in unmapped areas during the past 5 Myr, extrapolate the results back in time, and make corrections for differential subduction. The increase in the rate of floor production with time remains, nevertheless, even when these procedures are complete.

As for numbers, Steiner estimates that the average rate of ocean floor production over the past 165 Myr was $2.61\ \text{km}^2\ \text{yr}^{-1}$. He also concludes that the average subduction rate over the same period was only $1.88\ \text{km}^2\ \text{yr}^{-1}$ and that this average too conceals an increase with time. However, the procedure for obtaining the latter figure is, if anything, even more involved than that necessary to determine the average spreading rate; and therein lie two difficulties. The first is the question of credibility. The greater the degree of assumption, extrapolation and 'correction' there is in calculations of this sort, the less convinced the reader will be of the validity of the result and the greater will be the opportunity for picking holes in the argument. This certainly implies no criticism of Steiner, who has done the best he can with the limited data available. In the circumstances, however, it is bound to be difficult to convince those who are predisposed not to believe in Earth expansion.

The second problem, which may only be a special case of the first, is that the more complicated the procedure and the larger the number of mathematical processes involved, the greater will be the errors on the figures obtained. Thus Steiner estimates the errors to be ±15% on the areal production rate and ±20% on the subduction rate. The obvious conclusion to be drawn from the rates themselves is that over the past 165 Myr the area of ocean floor produced was 33% greater than the area destroyed, which interpreted in terms of an expanding Earth would imply a Jurassic palaeoradius of 5,668 km, or 0.89 of the Earth's present radius. The final error on the palaeoradius, however, is ±13%. Unfortunately, this is just large enough to allow the figures to be interpreted in terms of an Earth of constant radius even though, as Steiner points out, there is good reason for regarding 5,668 km as a maximum.

So Steiner has come up against the problem that has bedevilled most previous attempts to test the hypothesis of Earth expansion, namely, that the basic data have insufficient resolving power. Through no fault of his own, his analysis is unlikely to convert anyone to a belief in expansion, although equally it will do nothing to discourage those already converted. ☐

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review article

Quark confinement

R. L. Jaffe*

Nucleons and other subnuclear particles seem to be composed of more fundamental objects, the quarks, but bombardment of nucleons at increasing energy has failed to liberate quarks from the interior of nucleons. This has led to the development of theories in which quarks are of necessity permanently confined. Models of the dynamics of confined quarks, such as the 'MIT bag', have unified our understanding of subnuclear phenomena.

FROM time to time in the past the 'fundamental particles' of physics have been shown to be composites of particles yet more elementary. Molecules are composed of atoms, atoms in turn of electrons and a nucleus, the nucleus in its turn by protons and neutrons. Now there is impressive evidence that protons, neutrons, and the host of short-lived particles with which they interact strongly are composed of yet more fundamental objects called quarks. Quarks, however, have not been found free in nature; and if present theories are correct they never will be: they are thought to be permanently confined to the interior of the particles they compose.

This is a radical departure from the behaviour of other more familiar composite systems. It is relatively easy to separate an atom from a molecule, an electron from an atom or a proton from a nucleus. It is thought not merely to be difficult but actually to be impossible even in principle to remove a quark from inside a proton. This idea is rather young. It has been taken seriously only since early this decade. We have no fundamental explanation of quark confinement, nor an operational description consistent with relativity and quantum mechanics—still the foundations of modern physics. Nevertheless, in the past few years a possible theory of quarks has emerged which is aesthetically attractive and at least consistent with confinement; and an operational model incorporating confinement has been successful in describing many phenomena. Before describing these advances it is necessary to outline the events which forced the extraordinary notion of permanent confinement upon us.

The problem

Today's 'elementary' particles are of two types. There are a few 'leptons'—the electron and neutrino being the most familiar—and the photon, which interact only weakly with one another and with all others. So far these particles have shown no internal structure. They seem to be simple and may be fundamental. Because of their relative simplicity they are useful as probes of the other particles, the 'hadrons', which are certainly extremely complex. Hadrons are defined by the fact that they interact very strongly with one another. The best known are the proton and neutron. The well known effects of nuclear fission and fusion are manifestations of the extremely strong forces between hadrons.

It is the hadrons which are believed to be composed of quarks. In addition to the proton and neutron and the heavier nuclei there are hundreds of known hadrons. All of these others are very short lived. Hope has been abandoned that the hadrons themselves are elementary. Even the proton is not special. It happens to be stable only because there is no lower energy system for it to decay into. In fact the hadrons behave more like excited states of one particle, the hadron, than distinct entities.

With such a host of particles, classification becomes a major task. Hadrons, like atoms or nuclei, may be labelled by their quantum numbers. Examples familiar from atomic and nuclear physics are intrinsic angular momentum (called spin and labelled J) in units of Planck's constant $\hbar$; parity (P), which labels reflection symmetry, and takes values ± 1; and electromagnetic charge (Q) in units where the charge of the proton is $+1$. Quantum numbers are important to the extent that they are conserved by interactions. Charge, for example, seems to be absolutely conserved by all interactions. Hadrons carry other quantum numbers which have been painstakingly discovered over the years: 'baryon number', 'strangeness' and 'charm' are the names of ones of importance to us. Hadrons come in two broad categories: baryons and mesons, which are distinguished by their decays. All hadrons decay by emitting leptons, photons and/or other hadrons, the last of which in turn decay in the same manner. Baryons always leave a proton among their decay products. Their antiparticles, antibaryons, always decay eventually into antiprotons. Mesons either decay entirely into leptons and photons or into baryon–antibaryon pairs. To quantify all this, baryons are assigned 'baryon number' $+1$, antibaryons are assigned $B = -1$, and mesons are assigned $B = 0$. The decay patterns are equivalent to conservation of baryon number. No known interaction violates this conservation law.

The detailed significance of strangeness (S) and charm (C) need not concern us here. It is necessary only to regard them as measurable integer labels carried by hadrons, which are conserved in strong interactions but not in the weak interactions responsible for nuclear beta-decay. Quite early in the game it was observed that strange hadrons ($S \neq 0$) were systematically heavier than non-strange hadrons with otherwise identical quantum numbers. When hadrons with charm ($C \neq 0$) were discovered last year they were found to be heavier still. It is quite possible that there are other even heavier hadrons with other labels which have yet to be discovered. Quantum numbers like charge, strangeness, and charm which distinguish among mesons and among baryons are known generically as 'flavours'.

The proton and neutron have very nearly the same mass (about 938 MeV) and are quite similar in many other respects. They differ, of course, in their electromagnetic charge. All hadrons follow this pattern: they come in (small) families or 'multiplets' with very similar properties but different charges. The explanation (proposed long ago by Heisenberg[1]) is that the strong interactions are to a very good approximation 'charge-blind'. This symmetry, known as 'isospin' symmetry, describes the types of families and the similar properties of members of a family but does not determine which families exist. Why the

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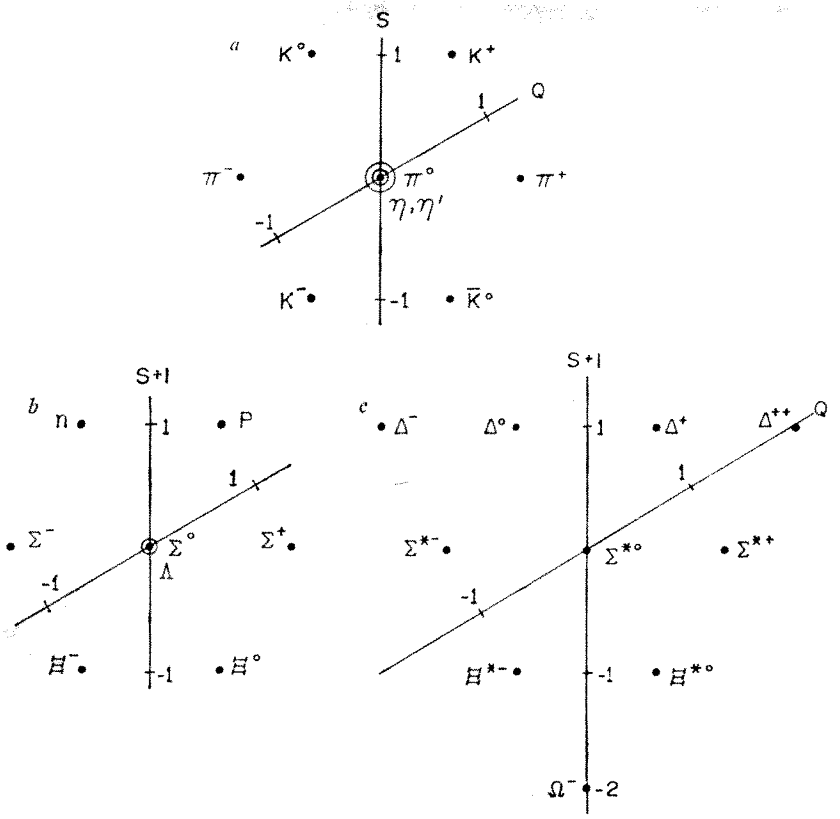


Fig. 1 Hadron multiplets: strangeness is plotted against charge using inclined axes to better display the symmetry. All hadrons in a multiplet have the same intrinsic spin and parity. Within a multiplet states with strangeness are systematically more massive. *a*, 0^- meson nonet; *b*, $\frac{1}{2}^+$ baryon octet; *c*, $\frac{3}{2}^+$ baryon decuplet.

proton–neutron form a doublet, while the π-mesons form a triplet (charges −1, 0, +1), and the Δ-baryons form a quadruplet (charges −1, 0 + 1, +2) is not explained by this observation.

After the discovery of strange hadrons in the early 1950s attempts were made to incorporate strangeness into the classification scheme. The idea was to assign hadrons to families with similar properties (in particular with the same spin and parity) but with different charge and strangeness. Strange hadrons weigh somewhat more than their non-strange siblings so the strong interactions are not blind to strangeness, but they are near-sighted enough to allow us to group particles into families anyway. The correct scheme was discovered by M. Gell-Mann[2] and Y. Ne'eman[3] in 1961. The arrangement of charges and strangeness within a multiplet and the number of states in the multiplet are closely related and are dictated by the mathematical formulation of this approximate symmetry. Some of the most prominent hadron multiplets are shown in Fig. 1. Before the advent of charm in 1976 all known mesons were found to come in multiplets of 9 (nonets) and all known baryons in groups of 1, 8 or 10 (singlets, octets or decuplets).

Still there was no explanation for this apparent multiplet structure. To borrow an anology from atomic physics, the scheme of Gell-Mann and Ne'eman is like the periodic table of Mendeleev. It classifies, but does not explain, the properties of hadrons.

The notion of quarks was introduced in 1964 independently by Gell-Mann[4] and Zweig[5] to explain why baryons come only in multiplets of 1, 8 and 10, and mesons in multiplets of 9. They proposed, for the purposes of classification, to imagine that hadrons are composed of three types of more fundamental particles—quarks[6]. Baryons were to be made of three quarks (Q^3), mesons of a quark and an antiquark $(Q\bar{Q})$. To get the hadron quantum numbers right quarks must have very odd quantum numbers themselves. If the proton is to have baryon number one, then quarks must have baryon number one-third. To give the correct electromagnetic charges for the hadrons quarks must have charges which are 2/3 or −1/3 times the proton's charge. No one has ever observed a particle with fractional charge or baryon number. In 1964 the idea was so radical that most physicists preferred to view quarks as theoretical conveniences—an efficient way of summarising the observed hadrons—not as real objects expected to be observed in the laboratory.

Gell-Mann's three quarks were named u, d and s (for 'up', 'down', names which arose from the formalism of isospin and 'strange'). The quarks' quantum numbers are listed in Table 1. To make mesons combine any quark and antiquark. There are nine combinations with a given total spin and parity just as observed. The quark content of a sample nonet is shown in Fig. 2. Baryons are somewhat more complicated. The families of 1, 8 and 10 are shown in Fig. 3. A bit of tedious mathematics would be required to explain why only these multiplets are allowed. By combining the spins of three quarks it is possible to make multiplets with total spin 1/2 or 3/2. Nature, however, is more selective: the octet containing the proton and neutron has no spin 3/2 counterpart; the spin-3/2 decuplet shown in Fig. 1 has no spin-1/2 analog. What forbids these other configurations? The answer lies in permutation symmetry. The characteristic shared by the allowed baryon configurations is that they are totally symmetric with respect to exchange of any pair of particles. Apparently, then, the quark model must be augmented by the rule that only symmetric configurations are allowed.

This raised a serious puzzle. Long ago Pauli[7] demonstrated a

Table 1 Quantum numbers of quarks

Quark	Charge	Baryon number	Strangeness	Charm	Spin	Mass (MeV)
u (up)	2/3	1/3	0	0	$\hbar/2$	0
d (down)	−1/3	1/3	0	0	$\hbar/2$	0
s (strange)	−1/3	1/3	−1	0	$\hbar/2$	300
c (charmed)	2/3	1/3	0	1	$\hbar/2$	1500

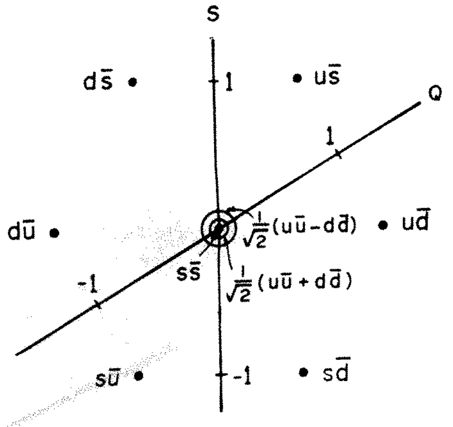
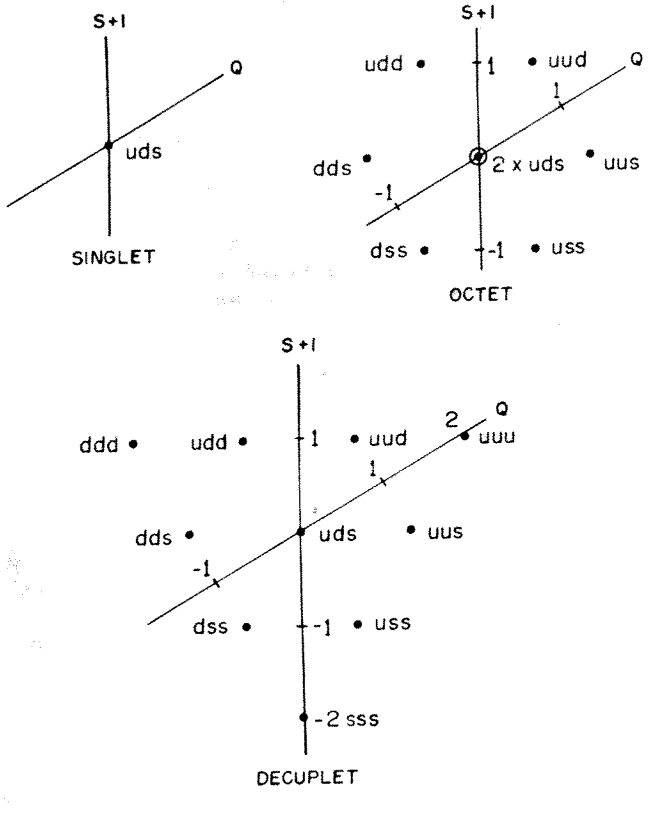

S
ds̄
1
us̄
Q
1
dū
$\frac{1}{\sqrt{2}}(u\bar{u}-d\bar{d})$
ud̄
-1
ss̄
$\frac{1}{\sqrt{2}}(u\bar{u}+d\bar{d})$
sū
-1
sd̄

Fig. 2 The quark content of a typical meson nonet. Compare Fig. 1*a*.

deep connection between symmetry and intrinsic spin: particles with integer spin (in units of $\hbar$) combine with each other only in symmetric configurations while those with half-integer spin combine only in antisymmetric arrangements. Quarks seemed to violate this. Early on this seemed yet another reason not to take the quark idea too seriously. Now it seems to be an essential clue to resolving the mysterious behaviour of quarks. We shall return to it shortly.

Over the years it has proved possible to account for a great variety of phenomena by supposing hadrons to be made of quarks[8]: the magnetic moments of the proton and neutron, photo-excitation of the proton, very high energy scattering of hadrons off one another, to name only a few. The flavour conservation laws observed in strong interactions reduce to the simple rule that the strong force never changes a quark's flavour. The strangeness of a bunch of hadrons is just the number of $\bar{s}$-antiquarks minus the number of s-quarks. Strong interactions may create or annihilate $s\bar{s}$-pairs but the total strangeness is conserved. Weak interactions are flavour changers: in the β-decay of a neutron, for example, ad-quark changes into a u-quark.

Fig. 3 The quark content of a typical baryon singlet, octet and decuplet. Compare Fig. 1*b* and *c*.



The major problem with the quark model was thought to be the failure of attempts to observe free quarks. Originally this was explained by assuming them to be very heavy (say thousands of MeV) and to be bound by very strong forces to make much lighter hadrons like the proton. To remove a quark from a proton would require restoring the binding energy and would be difficult. This is not 'confinement' as we now understand it but just 'binding' as in atomic physics on a grander scale. This picture was difficult to reconcile with the way the model had been applied: quarks were treated as free and light (roughly 1/3 as massive as the proton) and the properties of hadrons were obtained just by adding up the properties of quarks. Interactions generally modify the properties of particles, the stronger the interaction the greater the modification. If the quarks within a proton were bound with binding energy equal to several times their rest mass it seems uncanny that the unmodified quark properties should persist.

Quarks were only 'mathematical' particles anyway. It seemed premature to worry about these dynamical puzzles until someone had actually discovered a quark. During the late 1960s many quark searches were undertaken—none was successful. Finally a series of beautiful experiments[9] led by J. Friedman and H. Kendall of MIT and R. Taylor of SLAC begun in 1967 obtained indirect but persuasive evidence for the existence of quarks within the proton. The MIT–SLAC collaboration scattered high energy electrons off proton targets. The motion of electrons is effected by the local electromagnetic currents within the proton. Electrons do not interact strongly and are relatively structureless. Because of this they make excellent probes of hadrons. The greater the angle through which the electron scatters the shorter distances within the hadron it probes. The experiments showed that when scattered through large angles, the electrons behaved as though scattered elastically from light, spin-1/2 constituents within the proton. This behaviour is known as 'Bjorken scaling' after J. D. Bjorken of SLAC who first suggested such behaviour might be expected[10]. Similar phenomena are well known in other branches of physics[11]. Consider scattering of electrons off a nucleus. At very small angles the electron most often scatters elastically from the nucleus as a whole. At larger angles the scattering is predominantly inelastic, leaving the nucleus in an excited state or fragmenting it into a few pieces. At even larger angles the electron scatters nearly elastically from the individual protons in the nucleus. For this to occur the energy transferred to the proton must be large compared with its binding energy. So the proton is knocked out of the nucleus and could be observed propagating freely after the collision. If the proton had not been independently discovered long before such experiments were performed the behaviour of the scattered electron would have been taken as exciting evidence that the nucleus is composed of weakly bound charge 1 constituents—protons. The neutrons, being electrically neutral, would have been missed.

So it was with quarks. Electron scattering from a nucleus and from a proton are compared in Fig. 4. The observation of Bjorken scaling indicates that there are more fundamental, local bits of charge within the proton. These were called 'partons' by Feynman[12,13]. This series of experiments and subsequent similar neutrino–proton scattering studies suggest that the proton's 'parts' are quarks. In fact there is no serious alternative description of large angle lepton scattering. So hadrons are made of quarks. There might also be electrically neutral quanta (which would not show up in these experiments) to mediate the interactions between quarks. These are known generically as 'gluons', the 'glue' which holds the proton together.

It must be emphasised that the MIT–SLAC experiments showed the proton to be a rather loosely bound collection of quarks. At distances of the order tenths of a fermi within a proton (the proton's radius is about 1 fermi = 10^{-13} cm), there was little if any evidence for forces on the quarks. In short, everything was analogous to inelastic electron scattering off nuclei except that in the nuclear case protons are being knocked out of the nucleus while in our case quarks are not being knocked

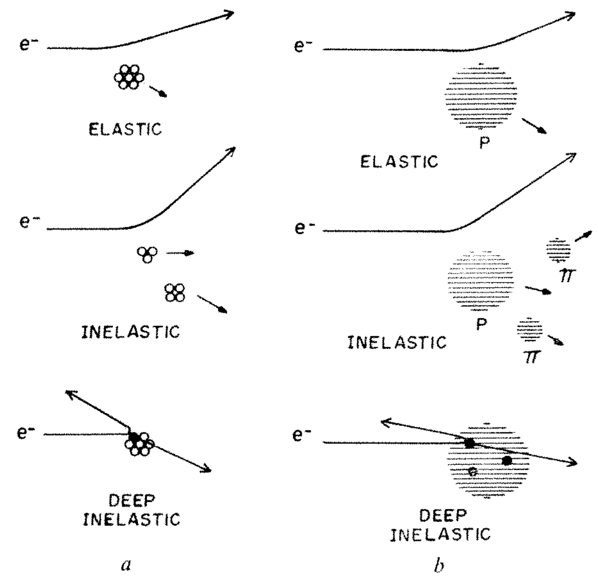


Fig. 4 A comparison of elastic and inelastic electron scattering off a nucleus (*a*) and a proton (*b*). In each case as the scattering becomes more inelastic, it becomes more sensitive to the local charge structure within the target. In case (*a*) when the energy transferred to the nucleus is much greater than a proton's binding energy, the electrons seem to scatter elastically from the individual protons. In case (*b*) the electrons eventually seem to scatter elastically from quarks within the proton.

out of the proton. Hadrons appear to be weakly interacting aggregates of light quarks which nevertheless can never be removed from the interior of the particles they compose.

In the years since the original MIT–SLAC experiments, evidence supporting the quark model has steadily accumulated. During the early 1970s neutrino scattering and electron–positron annihilation have emerged as significant new methods for the study of hadrons. Simple and elegant explanations of the phenomena observed in these new regimes have come from the quark model. Recently hadrons containing a new, heavier quark—the c-quark (for 'charmed')—have been discovered. The mass of the c-quark is thought to be roughly 1,500 MeV. The old quarks are much lighter: the u- and d-quarks may even be massless while the s-quark's mass is about 300 MeV. Because it is heavier the motions of the c-quark are easier to sort out. Quark model enthusiasts have been having a field day predicting (with great success) the properties of new 'charmed' hadrons composed of c-quarks together with the old u, d and s-quarks[14].

The picture of relatively weakly interacting but permanently confined quarks permeates particle physics today. Unfortunately application of the picture to different problems rest on different *ad hoc* assumptions whose relationship to one another is often unclear. It is not a unified picture because we lack a unified theoretical description of confinement. At best, one would like to have a derivation of confinement from some deeper theoretical notions. At least one could hope to have an operational description of confined quarks consistent with the basic requirements of relativity and quantum mechanics. Recently there has been progress in both directions. Many of the ingredients of a fundamental theory of quark confinement perhaps have been discovered. These will be reviewed in the next section. The fundamental work unfortunately remains incomplete because the candidate theory cannot be solved: confinement cannot be demonstrated and the properties of hadrons cannot be calculated.

The theory

We have come very far in understanding the origins of the bizarre behaviour of quarks. The first step towards a solution was the realisation that hadrons possess a secret—a quantum number which is essential in determining quark interactions but which never manifests itself openly in nature. Suppose in the days of atomic physics it had proved impossible to ionise neutral atoms, so only electrically neutral atoms were known. Eventually someone would have guessed that the constituents of atoms carry a hidden quantum number (this should not be confused with a 'hidden variable' which arose in a famous unsuccessful attempt to explain in purely classical terms the indeterminacy usually attributed to quantum mechanical effects)—electrical charge—which dictates the way they are put together in atoms but is never openly manifested in nature. In the case of quarks this hidden quantum number is called colour. It has nothing to do with ordinary visual colour, particle physicists just seem to like familiar names for their rather abstract notions. There are three possible quark colours which we may take to be 'red', 'green' and 'blue'. Each flavour of quark (u, d, s or c) comes in each of the three colours, but hadrons are always combinations of quarks with no net colour. They are colour neutral. (The reader may well wonder how fundamental quarks are now that we have twelve of them. What one calls a new particle is perhaps a matter of semantics. A particle with spin J has $(2J+1)$ different spin orientations which are not considered distinct objects. We likewise may not wish to call each different colour orientation of each flavour of quark a different particle[15]. In any case quarks will be more fundamental than hadrons if their interactions admit a simple, point-like description.)

Colour was originally introduced by Han and Nambu[16] (another early version of the idea is due to Greenberg[17]) to resolve the symmetry mystery mentioned earlier: quarks in baryons seemed to occur only in symmetric configurations although Pauli had shown spin-1/2 particles must occur in antisymmetric arrangements. The solution was simple: the baryons are indeed symmetric in all the observed labels: spin, flavour and spatial configuration, but each quark carries an additional label—colour—and the baryons are antisymmetric arrangements in the colour label. So much for the symmetry mystery. Introducing a new quantum number is risky: the number of possible arrangements of quarks is greatly increased. Should all of these be observed in nature? In fact only a small selection of all conceivable arrangements of colour, flavour, spin and spatial quantum states have been found: all mesons may be classified as a quark and antiquark ($Q\bar{Q}$) of the same colour; all baryons may be classified as three quarks (Q^3) made asymmetrical in colour. Remarkably these are the only combinations of $Q\bar{Q}$ or Q^3 which are colourless. (Certain combinations of coloured quarks have no net colour just as certain combinations of charged electrons and protons have no net charge.) Colour could be introduced into the theory without predicting a vast, unwanted increase in the number of baryons and mesons if accompanied by a rule: all hadrons must be colour neutral.

There is an intimate relation between colour and confinement. A single quark, along with its fractional charge and baryon number, necessarily carries colour. So does any arrangement of two quarks, all of which also have fractional charge. However, a quark and an antiquark, which according to Table 1 have ordinary (integer) charges, also turn out to be the smallest arrangement of quarks with no net colour. The same goes for baryons—groups of three quarks have integer charge and baryon number and, remarkably, may be grouped into colourless configurations. In fact it can easily be shown that any combination of quarks and antiquarks which is colourless also has ordinary quantum numbers. Having learned this we can restate the confinement problem rather elegantly: quarks carry a new quantum number, colour, but for some reason all hadrons are colourless. Quark confinement has been reduced to colour confinement. Quarks must have interactions which select out colourless configurations. The case for colour as it stood in 1973 was summarised rather persuasively by Fritzsch, Gell-Mann and Leutwyler[18], who also proposed a candidate for the actual quark–quark interaction (though the interaction itself seems to have been discovered independently by several workers[19,20].)

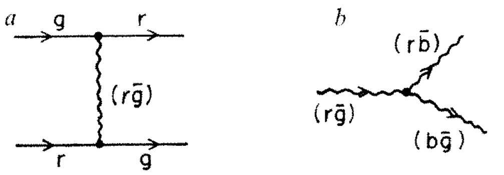


Fig. 5 A sample of interactions in quantum chromodynamics: *a*, red and green quarks scatter by exchanging a quantum of red-greenness; *b*, a red-green gluon emits a red-blue gluon and changes thereby into a blue-green gluon.

The theory of the interactions of electrons and photons, known as quantum electrodynamics (QED), is the most precisely verified theory in science. It is the model for construction of a theory of interactions consistent with both relativity and quantum mechanics. For some years there had been interest in generalisations of quantum electrodynamics known as Yang–Mills[21] theories. QED is based on one kind of charge (electric) interacting with one kind of massless vector field (the electromagnetic field, whose quantum is the photon). In 1954 Yang and Mills formulated a theory of several charges interacting with many vector fields. The novel ingredient was that the vector fields carry the charges (in contrast to QED since the photon is neutral). Thus a particle could change its charge by emitting a field quantum which carries the compensating charge. The theory requires the quanta of the many vector fields to be massless. As in QED there are both electric and magnetic forces between charges. The Yang–Mills theory was not applied to strong interactions during the 1950s and 1960s because there are no massless strongly interacting particles. The possibility of confinement changes all this: the forces between quarks might be of the Yang–Mills type so long as the massless vector quanta (gluons) along with the quarks are permanently confined.

To return to the work of Fritzsch, Gell-Mann and Leutwyler, they supposed that the forces between quarks were of the Yang–Mills type described above. The colour quantum number plays the part of the charge. Quarks interact through colour electric and magnetic fields. With three quark colours it is necessary to have eight massless vector fields—'coloured gluons'. Eight gluons are required in order to maintain conservation of the colour quantum numbers and for all colours to be on an equal footing. A red quark may change into a green quark by emitting a quantum of red-greenness, that is, a red-green gluon. There are nine combinations of colours like red-green, but one combination is actually colourless and does not enter the interaction scheme. Figure 5 shows a sample of the basic interactions of the theory. Because the gluons themselves are coloured they interact with one another much like quarks. This theory turns out to be the only natural generalisation of quantum electrodynamics to a non-trivial group of conserved charges. For this reason it has become known as 'quantum chromodynamics' or QCD.

The case for QCD became compelling later in 1973 when D. Gross and F. Wilczek[19] and H. D. Politzer[20] independently discovered a deep connection between QCD and Bjorken scaling. Generally forces become stronger at short distances. Everyone knows Coulomb's law: $F = e_1 e_2/r^2$ provides a force which increases as charges are brought together. In fact the forces of electromagnetism are even stronger at short distances than Coulomb's law would indicate. At very short distances where relativistic and quantal effects are essential the effective charge, *e*, actually changes. It has long been known that in QED the effective charge increases at short distances. The MIT–SLAC electron scattering experiments however showed that quark–quark interactions become negligible at short distances within the proton. Gross and Wilczek and Politzer found that the gluon-mediated interactions in QCD, do grow weaker at short distances and vanish asymptotically at zero separation as required. No other realistic theory has this property of 'asymptotic freedom'. QCD is singled out as a candidate theory of the strong interactions.

The Yang–Mills theory of coloured quarks and gluons contains all of the ingredients believed to be required of a fundamental theory of the strong interactions. Furthermore it is very precisely formulated. Nevertheless it has not been possible to address the basic question: does this theory lead to the confinement of quarks? The reason for this predicament lies in the deep distinction between formulating a theory of interacting fields and applying it to most physically interesting problems. Theories are formulated 'locally', that is, the nature of the interaction between fields, here quarks and gluons, is specified at an arbitrary point in space and time. Hadrons are, however, extended regions of space in which quark and gluon fields interact with one another constantly. The comparatively simple local interactions can give rise to an arbitrarily complex global structure for a hadron.

Some approximation must be made. Quantum electrodynamics is the only example we have of a theory which is consistent with both relativity and quantum mechanics and for which approximate solutions can be found. The key to approximation is the weakness of the electromagnetic force. The dimensionless measure of its strength is the 'fine-structure constant' $\alpha = \varepsilon^2/\hbar c$ (c is the speed of light, ε is the electronic charge and $\hbar$ is Planck's constant). Since α is about 1/137, things may be calculated perturbatively. To a first approximation there are only free electrons and photons. To next order two electrons have a small probability amplitude ($\sim 1/137$) to interact by the exchange of a photon, and so on. The forces between particles generated in this fashion characteristically fall off with increasing distance. Systems of interacting particles can be assembled by bringing the components together from large distances where their interactions are negligible.

This won't work for a theory of quarks. They are supposed to be permanently confined. There is no sensible limit in which the quarks can be treated as isolated particles. Over very short distances inside a proton quarks interact quite weakly because of 'asymptotic freedom' and a form of perturbation theory can be used to calculate their behaviour. But only a few properties of hadrons are sensitive exclusively to what happens at short distances. Most involve distances of the order of the proton's radius where interactions must begin to become strong if quarks are to be confined. The usual perturbative treatment begins with free quarks and gluons which, if the programme is successful, must miraculously disappear in the finale.

A new approximation scheme is needed. Efforts in this direction have yet to be successful but the attempts themselves have introduced many exciting new ideas into theoretical physics. One approach is to alter the structure of space–time in order to make calculations more convenient. In this work, pioneered by K. Wilson[22] and by J. Kogut and L. Susskind[23], space or both space and time are replaced by a lattice of discrete points. To lowest order quarks sit on a single lattice site. To higher orders quarks move from site to site: the hadron spreads out. It is not difficult to show that in this approach to QCD quarks can never be separated from one another. Of course, space–time is not a lattice. There is evidence for its continuity down to distances of the order of 1/100 the proton's radius[24]. The burden on the lattice approach is to determine whether its predictions are consequences of the original quark gluon theory or artefacts of approximating space–time as a lattice. This dilemma remains unresolved today.

There has been much excitement recently concerning non-perturbative solutions to field equations. Often classical theories of interacting fields admit exact solutions where the field configurations are in no sense small perturbations about the trivial solution—empty space. According to their properties such solutions are known as 'solitons' or 'instantons'. Theories which admit these solutions seem to be qualitatively different from those which do not. Ordinary quantum electrodynamics seems not to. Quantum chromodynamics does, and it is thought that the existence of these solutions is associated with confinement.

An association like this occurs in the theoretical description of magnetic fields in superconductors. When metal in a magnetic field is cooled and becomes superconducting the lines of

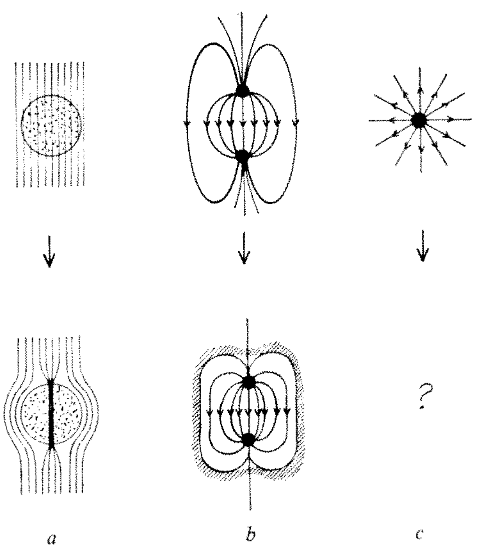


Fig. 6 A superconductor analogy for confinement. *a*, When an ordinary metal in a uniform magnetic field is cooled and becomes a superconductor the magnetic flux lines are excluded from the superconducting region though some may be trapped in 'flux tubes' (Meissner Effect); *b*, quark confinement may be due to a colour-electric Meissner Effect in which the vacuum excludes colour electric flux. A quark–antiquark system is confined to a 'bag' but a single quark cannot exist since its flux lines have no place to end and cannot stretch out to infinite distance.

magnetic flux are expelled from the interior of the superconductor, though some may be trapped within tubes of 'normal' metal within it. This effect, illustrated in Fig. 6*a*, is known as the Meissner effect[25]. The magnetic field lines are, after a fashion, confined. Although this strange behaviour is caused by the interactions of the electrons within the metal, it cannot be obtained by treating them perturbatively. In an ordinary metal electrons move about rather freely much like a gas in a box. In superconductors there is a configuration of interacting electrons which is radically different and lower in energy than the free 'gas' configuration[26]. Since it is lowest in energy it is the preferred (ground) state of the system. All of the bizarre properties of superconductors are attributable to this non-perturbative ground state.

Similar non-perturbative solutions to Yang–Mills theories were found by Polyakov and his collaborators[27]. These and other recently discovered non-perturbative solutions to Yang–Mills theories have excited intense interest. As yet no one has succeeded in establishing a connection between them and the confinement problem. The Meissner effect seems much simpler than QCD.

The analogy with superconductivity can be pushed further. S. Mandelstam has particularly emphasised this point of view[28]. A superconductor expells magnetic flux lines. Suppose empty space (the vacuum) expells colour-electric flux lines. There is a Gauss's law in QCD as in electromagnetism. It requires that lines of colour-electric flux, which begin and end on coloured quarks and gluons, cannot be cut. The colour charge within a given region of space can be measured by surrounding the region with an imaginary surface and counting the number of colour flux lines crossing the surface. If, for some unknown reason, the vacuum expells colour flux lines then all coloured objects and all their attendant flux lines must be confined to some 'normal' region. An isolated quark could not exist since it is the source of flux lines which would have to stretch out for infinite distances. Only configurations with no net colour could exist since they are not required to have flux lines extending to infinity. If this could be shown then QCD would give us an explanation for quark confinement. The 'normal' and 'colour–electric superconducting' states are illustrated in Fig. 6*b* and *c* for a quark–antiquark pair and for a single quark. Perhaps the non-perturbative solutions of QCD will have the effect of making the vacuum behave towards colour–electric flux the same way that a superconductor behaves towards magnetic flux. As present this is merely speculation.

The model

Long before the modern explanation of superconductivity and the Meissner effect were known, Ginsberg and Landau made a model to describe superconductivity without attempting to derive it[29]. Since confinement seems to be at least as hard a problem, my collaborators and I at MIT have taken a similar approach to it[30]. It turns out to be easy to write down a simple and elegant model incorporating confinement. Our approach is more radical than that of conventional quantum field theory. Rather than starting with quarks and gluons everywhere, and then trying to confine them, we start by assigning coloured fields only to the inside of hadrons—since that is where they seem to be. This makes it necessary to distinguish the space inside a particle from that outside it—the hadron must be an 'extended', geometrical object. The shape of a hadron cannot in general be fixed. Rigid objects are not consistent with relativity. If an object is hit at a point on its surface it is not possible for the object to recoil rigidly because other points on the surface would have to have learned of the impact instantaneously. Relativity requires that no information travel faster than the speed of light. Each point on the boundary must be free to move. The equations which describe the motion of the boundary of an extended object must be local in order not to violate relativity.

It is possible to distinguish an otherwise empty region of space in a way consistent with relativity by subjecting the boundary of the region to a constant pressure, B where B may be thought of as a pressure exerted by otherwise empty space on the hadron. There is a simple, classical picture of the dynamics of such an object. A hadron is like a bubble of gas in a uniform, isotropic perfect fluid. The dynamics of a gas bubble is determined by the balance between the external pressure of the fluid and the thermodynamic pressure of the confined gas. A hadron is such a bubble containing only a few quanta instead of the vast number of particles in a macroscopic bubble. Quanta confined to a region of size $-\Delta x$ have—by Heisenberg's uncertainty principle—a momentum $\Delta p \sim \hbar/\Delta x$ and therefore exert a counter-pressure. This quantum pressure keeps the hadronic region open by pushing against the external pressure B. The pressure B fixes the size of hadrons. For protons to be of radius 1 fm (1 fm $= 10^{-13}$ cm), B is required to be about 60 MeV fm^{-3} (or 8×10^{28} atmospheres). This extended region of space is called a 'bag'. Since the model was developed at MIT is has become known as the MIT bag model.

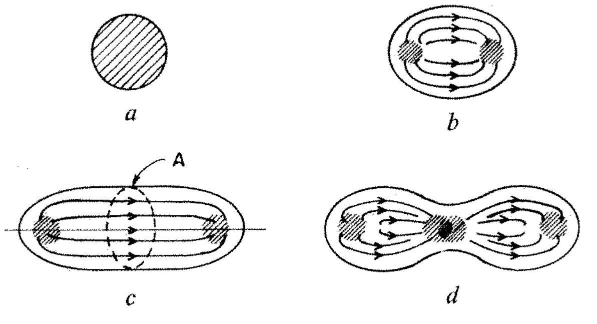
Quarks and gluons are the quanta of coloured fields. What about a one quark bag which would be coloured and have fractional charge? States like this never appear in the model: by construction, colour electric fields, like quarks, exist only inside the bag; but by the analog of Gauss's law, colour electric flux lines must emanate from a bag with net colour. The only consistent solution is for all bags to have no net colour. In fact an infinite energy barrier prevents a colour neutral bag from fissioning into two coloured bags. The situation is illustrated in Fig. 7. Suppose in a colour singlet bag, the colours C and $-$C are separated by a finite distance. The colour electric field lines which connect the charges C and $-C$ exist only in a finite volume V since (in this model) it costs an energy $BV > 0$ to create the space which carries them. Hence at a point between the charges, the fields span a finite cross section A and by Gauss's law, $C = EA$ where E is the mean colour field strength in the cross section. The energy stored in a colour electric field charges as half the square of the field strength, therefore the energy per unit length is $\frac{1}{2}E^2A = \frac{1}{2}C^2/A$. Separating the colours by a distance l costs an energy $\frac{1}{2}C^2l/A$. Fission requires $A \rightarrow 0$ and $l \rightarrow \infty$; it requires an infinite energy to separate colours. Thus colour-, and hence quark-confinement is automatic and comprehensible in this model.

The particular virtue of this approach is that is offers the hope of calculating hadron properties perturbatively[31]. Quarks are arranged into colourless states *ab initio*. The agent responsible for this—a universal pressure—is simple in principle. With such a simple starting point it has proved possible to reconcile some of the apparently unrelated applications of the quark model with one another. Studies of the model reveal two broad classes of hadron which may be called 'spherical' and 'deformed'[32]. Hadrons made of u, d and s quarks with small amounts of angular momentum form spherical bags. To lowest order such a hadron (the proton for example) is a bag of free quarks (as seen in the MIT–SLAC highly inelastic electron scattering experiments)[33]. To next order, the gluon mediated interactions modify the masses and other properties of the hadrons[31]. If, on the other hand, the hadron contains heavy quarks like the newly discovered charmed quark or if it possesses large amounts of angular momentum, it will deform. Deformed hadrons are actually simpler to picture. One is shown in Fig. 7*b*. Consider a meson made of charmed quark and antiquark. Heavy quarks move about less and exert little pressure, but the bag does not collapse around the heavy quarks because they are sources of colour flux lines. The colour fields exert pressure perpendicular to the lines of electric flux. The bag therefore deforms about the flux lines connecting the quark and antiquark. Angular momentum has the same effect; centrifugal force throws quark and antiquark apart and leads to deformation. Light quarks with little or no angular momentum are smeared out throughout the bag. On the average there is no source for colour flux and therefore no axis for deformation.

Detailed calculations of hadron properties have been carried out with the model[31–34]. One example will suffice to illustrate the sort of success which it has had[34]. The masses of spherical hadrons are determined by the quark–gluon dynamics in terms of two fundamental parameters: B, which sets the scale; and a 'coupling constant', α_c which measures the strength of the quark–gluon interaction. The same parameters determine the masses of deformed hadrons although the basic dynamics is quite different. In deformed hadrons the glue is present in lowest order and balances the pressure B, while in spherical hadrons only quarks are present in lowest order and gluon interactions cause small modifications in higher order.

Deformed hadrons have long been known to form families with different angular momentum and mass but otherwise similar properties. In nuclear physics similar families are called rotational bands. In subnuclear physics they are called 'Regge trajectories'. Hadrons on the same Regge trajectory have a remarkably simple relation between mass and angular momentum: $J = \alpha' M^2 + \alpha_0$, where J is the total angular momentum of the hadron, M is its mass and α' and α_0 are called the Regge 'slope' and 'intercept' respectively. One of the best known Regge trajectories is shown in Fig. 8. Experimentally it is found that the Regge slope α' is the same for both baryons and mesons and equals 0.9 GeV^{-2}.

Fig. 7 Hadron dynamics. *a*, A colour neutral spherical meson; *b*, energy is delivered to the meson causing the quark and antiquark to move apart; *c*, colour–electric flux lines connecting quark to antiquark prevent separation; *d*, eventually the energy density in the region between the quark and antiquark becomes sufficient to enable another $Q\bar{Q}$ pair to materialise. These provide new sources and sinks of colour electric field. The system shown in (*d*) will soon fission into two colourless mesons.



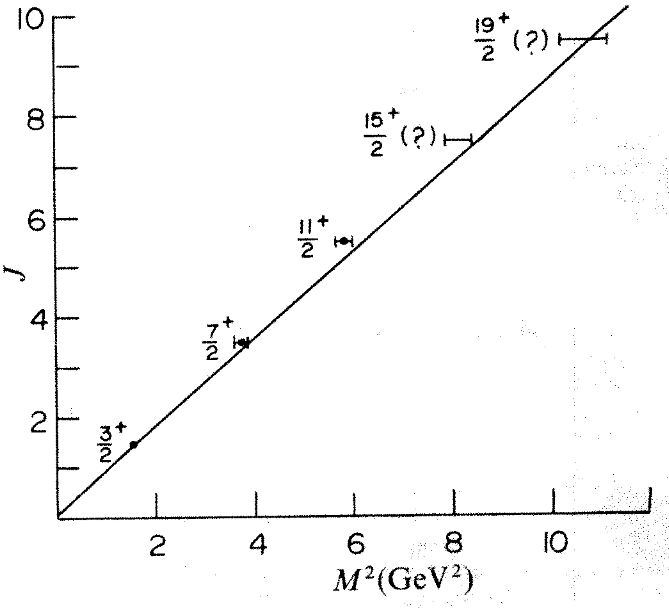


Fig. 8 The Δ-Regge trajectory: the points represent observed hadrons believed to be excitations of the Δ-baryon (see Fig. 1).

In the bag model hadrons with much angular momentum do obey the relation $J = \alpha' M^2 + \alpha_0$ and α' is the same for both baryons and mesons. The universality of α' is natural. The mass of a spinning hadron is determined by a dynamic balance of the gluon field pressure (involving α_c) and the universal pressure B. A spinning meson has opposite colours on either end. A spinning baryon has two quarks on one end and one on the other. The two quarks, however, have antiquark colour in order to combine with the remaining quark to make a colourless hadron. Thus the colour fields in a spinning meson and baryon are the same making α' universal. α' is found to be a simple function of α_c and B. To lowest order: $1/\alpha' = \pi^{3/2}\sqrt{8\alpha_c B}/3$. Remarkably, if α_c and B are adjusted to fit the masses of light, spherical hadrons, then α' comes out 0.88 GeV^{-2}. Two heretofore disjoint aspects of hadron phenomenology are therefore only different reflections of the same underlying physics.

At present the bag model has some important limitations. For example, it is not known whether it can be made consistent with quantum mechanics. All the applications discussed in the literature have made use of a semi-classical approach similar in spirit to Bohr's treatment of the hydrogen atom. In general, relativistic field theories are known to be consistent with quantum mechanics only when studied order by order in ordinary perturbation theory. But as we have noted, perturbation theory has little or nothing to say about confinement. The bag model abandons conventional perturbation theory in search of a starting point closely related to the observed phenomena of confinement. The price seems to be the loss of much of the useful conceptual machinery of quantum mechanics.

Subnuclear physics is in a rather peculiar state at prese
There is wide agreement as to the ingredients in a prospec
theory of hadrons: quarks, colour, gluons and the Y
Mills interaction, to name the most obvious. Yet there

precise, unequivocal and successful calculations of anything to bolster our faith in the theory. In QCD it is not even clear where to begin. If confinement is put in by hand by introducing the bag pressure B then many approximate calculations are possible. The resulting model is especially successful at understanding qualitative features of hadron behaviour. But even having abandoned the attempt to derive confinement one is still left with an extremely complex dynamical system which defies rigorous treatment and quantisation. It is not surprising that our description of hadrons remains in large measure incomplete. Nothing like the permanent confinement of quarks has been encountered before in the study of the structure of matter. Old approximation schemes fail, new ones develop slowly.

What is surprising is the emergence of a candidate theory of hadrons so soon after the realisation that quarks may be permanently confined to the interior of hadrons. It is particularly remarkable since none of the basic ingredients in the theory has ever been observed directly. Instead their existence has been inferred by brilliant and subtle qualitative analyses. The discovery of colour by way of the quark symmetry puzzle and the necessity of gluons and Yang–Mills interactions to understand the paradoxical 'free yet confined' behaviour of quarks are cases in point. The theory which has emerged is very precisely formulated and tightly constrained despite the rather indirect way its ingredients have been established. It offers the hope of explaining hadron properties in precise detail although at present only approximate calculations are possible. It is also very pretty, at least to particle physicists.

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articles

The location of the polio genome protein in viral RNAs and its implication for RNA synthesis

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Evidence is presented that a small protein (VPg) is covalently attached to the 5′-terminal oligonucleotide VPg-pU-U-A-A-A-A-C-A-Gp of the polio genome, to nascent strands of the polio replicative intermediate and to poly(U) of minus strands. A model of polio RNA replication is proposed implicating VPg in initiation of RNA synthesis, possibly as primer.

THE 5′-terminal structure of a ribonucleic acid may reveal mechanisms involved in the biosynthesis of that RNA. For example, the 5′ end of coliphage RNA is a nucleoside triphos- indicating that initiation of phage RNA synthesis occurs genomes of plus strand RNA viruses of eukar- e other hand, are 'capped' at the 5′ end (5′)Np . . . which is a post-transcriptional date, two exceptions to this group are known: f satellite tobacco necrosis virus which termin- G-U (ref. 3), and genome RNA of picornaviruses neither a terminal triphosphate nor a cappin- group. In fact, previous efforts to identify any 5′ end of picornavirion RNA were unsuccessful[4–9]. Recently, we have identified a low molecular weight protein (called VPg) covalently linked to polio virion RNA and have suggested that this protein may be attached to the 5′ end of the genome[10,11]. In this communication we present experimental evidence for the association of the protein with the 5′ end of virion RNA. We also show that VPg is bound to nascent strands of the polio replicative intermediate and to the 5′-terminal poly(U) of polio minus strands. This novel structural feature of poliovirus RNAs permits us to suggest a model of replication which implicates the protein in the initiation of RNA synthesis.

Genome-linked protein VPg at the 5′ end of virion RNA

The location of VPg within the viral genome can be determined by comparing the number of phosphates remaining on the nucleotidyl-protein obtained after digestion of the RNA with the enzymes RNase T2 or nuclease P1. As previously shown, digestion of purified virion ^{32}P-RNA with RNase T2 yields mononucleotides and a small protein of approximately 6,000 molecular weight (called T2-VPg), which is linked to pUp (ref. 11). As illustrated in Fig. 1*a*, this nucleotidyl-protein, [$(aa)_n$-ZpUp, where Z is the hypothetical linkage group between the protein and nucleotide], will contain two phosphate groups independent of its location on the RNA. Nuclease P1 is an endonuclease which yields nucleoside 5′-phosphates[12]. When virion RNA is cleaved at low enzyme concentrations (0.4 mg ml^{-1}, 2 h at 37 °C), the linkage between Z and pU is stable. As illustrated in Fig. 1*a* the nucleotidyl-protein produced by nuclease P1 (P1-VPg) would be predicted to contain only one phosphate if it were terminally bound and at least two phosphates if it were internally bound.

The isolation of P1-VPg can be seen in Fig. 1*b* which shows that the nucleotidyl-protein appears in the flow-through fractions of the ion-exchange column. T2-VPg appears at the same position in this column[11]. P1-VPg and T2-VPg also comigrate by electrophoresis on polyacrylamide gels (for conditions, see ref. 11). Analysis of the number of phosphate groups on P1-VPg required proteolysis due to the affinity of VPg to surfaces[10]. Accordingly, we digested ^{32}P-P1-VPg with Pronase which yielded a single phosphorylated product ^{32}P-P1-Y, with a net charge of −1 at *p*H 5 (Fig. 1*c*). P1-Y is resistant to alkaline phosphatase (data not shown) indicating the absence of a phosphomonoester. Isolation of the proteolytic product of ^{32}P-T2-VPg is shown in Fig. 1*d*. ^{32}P-T2-VPg has a net charge of −2 at *p*H 5 (ref. 11). Half of its label is sensitive to alkaline phosphatase (data not shown). Snake venom 3′-exonuclease yields pUp from T2-Y and pU from P1-Y (Fig. 2*a*, *b*). The data are compatible with ZpU being the structure of P1-Y. We therefore conclude that P1-VPg has the structure $(aa)_n$-ZpU and originated from the 5′ end of the viral RNA (Fig. 1*a*). The covalent attachment of a protein to the 5′ end of genome RNA represents a novel feature in the molecular biology of viruses.

Prolonged digestion of ^{32}P-T2-VPg with Pronase occasionally yielded T2-Y and a by product (T2-Y′) in low yield, which migrates faster than T2-Y during electrophoresis on Whatman 3MM paper. Incubation with aminopeptidase converts T2-Y quantitatively to T2-Y′ (Fig. 3). T2-Y′ yields pUp with snake venom exonuclease (not shown) as does T2-Y and is therefore a derivative of T2-Y from which presumably an amino acid or another ligand has been removed. The structure of T2-Y′ (Z′pUp), which harbours the linkage between VPg and poliovirus genome, is currently under investigation.

Isolation of a 5′-terminal, protein-linked oligonucleotide

To isolate a 5′-terminal fragment produced by RNase T1, we took advantage of the fact that VPg is positively charged as it migrates toward the cathode during paper electrophoresis at *p*H 3.5 or *p*H 7.5 (ref. 10). Thus, the protein-linked fragment would be the only oligonucleotide in an RNase T1 digest whose net charge would be changed by Pronase treatment permitting a 'shift out' experiment originally proposed by Tomlinson and Tener[13]. First, we separated an RNase T1 digest of virion [amino acyl-^{3}H]RNA by ion-exchange chromatography in 7 M urea (data not shown). Tritium counts were found only in a peak containing trinucleotides, an observation suggesting that the 5′-terminal oligonucleotidyl-protein has a net charge of −4. This was confirmed by repeating the experiment with virion ^{32}P-RNA (Fig. 4*a*). Material in peak −4 was desalted and digested with RNase T2 which yielded T2-VPg (Fig. 4*b*). The nature of this T2-VPg was verified by Pronase and nuclease digestion as described in Figs 1*d* and 2*a*. When the material in peak −4 (Fig. 4*a*) was digested with Pronase, a new oligonucleotide with increased negative charge was produced whereas the trinucleotides remained unchanged (Fig. 4*c*). The shoulder of the peak (⩾ −10) in Fig. 4*c* indicated to us heterogeneity of the oligonucleotide, the extent of which changed from experiment to experiment. The material in peak (⩾ −10) was pooled and separated into two spots ('oligo I' and 'oligo II') by two-dimensional gel electrophoresis (data not shown; see ref. 14). 'Oligo I' (which we found to elute ahead of oligo II from the ion exchange column; Fig. 4*c*) and 'oligo II' were recovered from the gel and characterised by further enzymatic analysis (see legend to Fig. 5). Separation of digestion products (Fig. 5) revealed that the oligonucleotides differ only in their amino acyl component resulting from overdigestion with Pronase: the nucleotides of oligo I are linked to Y, those of oligo II are linked to Y′. On the other hand, we repeatedly observed that the amounts of label in the digestion products of the 5′-terminal oligonucleotide disagreed with the expected yields (Table 1). For example, the radioactivity found in Y was

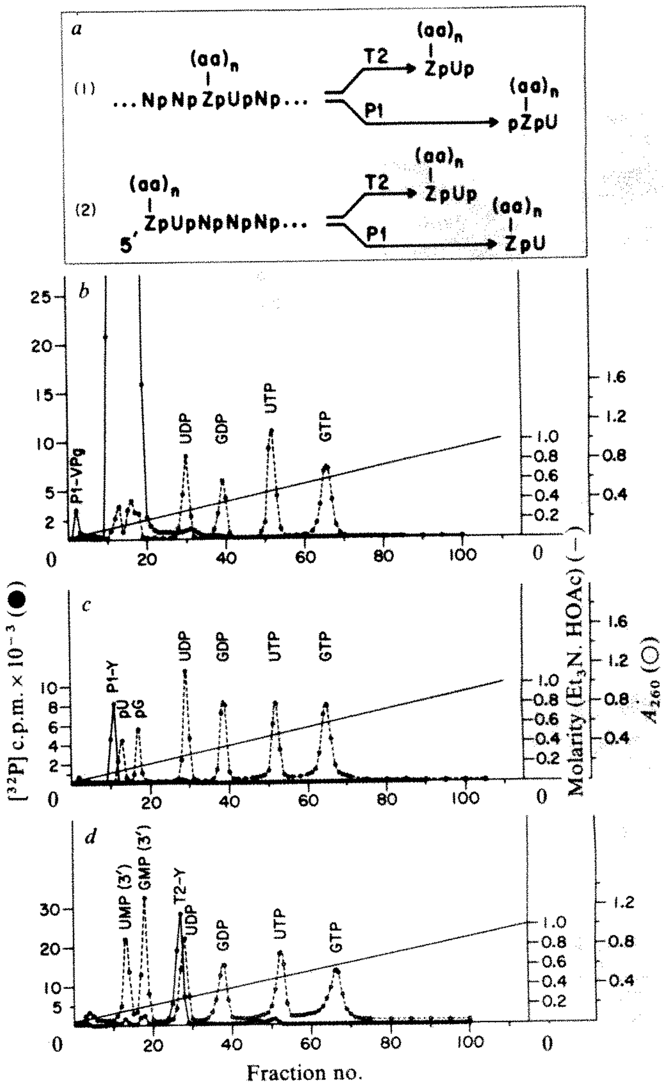

Fig. 1*a*, Hypothetical cleavage scheme for poliovirus RNA. The genome-linked protein may be bound to the RNA (1) internally or (2) terminally through a linkage group Z which has not yet been identified[11]. If internal, Z would be a nucleoside. If terminal, Z could be a nucleoside, one or more amino acids, or any other linkage group. Digestion of the RNA with RNase T2 or nuclease P1 would yield different nucleotidyl-proteins (T2-VPg or P1-VPg) which can be identified through the difference of their phosphate esters. *b–d*, Separation of digestion products of poliovirion RNA by column chromatography at pH 5 in the presence of marker nucleotides. Details as described in ref. 5. *b*, 8×10^7 c.p.m. ^{32}P–RNA were digested with 20 μg nuclease P1 (Yamasa) in 50 μl of 0.05 M ammonium acetate, *p*H 6, for 2 h at 37 °C. 8×10^3 c.p.m. nucleotidyl-protein, P1-VPg, were recovered by lyophilisation which is 52% of the theoretical yield (yield was corrected as explained in Table 1). In other experiments the yield was as high as 75%. *c*, ^{32}P–P1–VPg was digested in 200 μl of 0.05 M Tris-HCl, 5 mM sodium phosphate, *p*H 8, 0.1% sodium dodecylsulphate, with 10 μg Pronase (Sigma Protease type VI) for 20 h at 37 °C. *d*, Digestion of ^{32}P–T2–VPg with pronase as in Fig. 1*c*. P1–Y and T2–Y were recovered by lyophilisation.

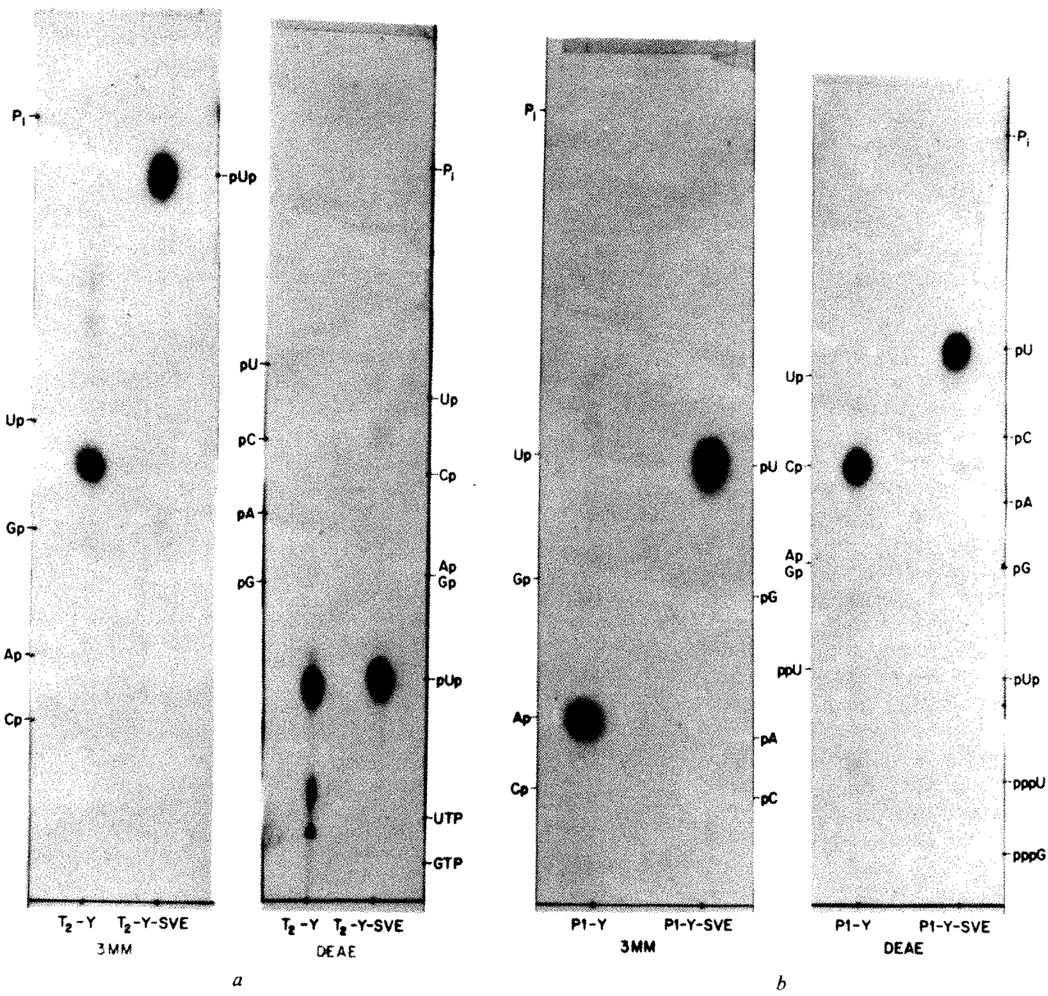

Fig. 2 Paper electrophoresis of ^{32}P-T2-Y and ^{32}P-P1-Y (see Fig. 2*d*, *c*) and their digestion products with snake venom 3′-exonuclease (SVE). *a*, T2-Y and *b*, P1-Y were treated with 0.5 μg purified exonuclease (gift from Dr M. Laskowski, Sr.) in 10 μl 0.02 M Tris-HCl, *p*H 7.5, 0.005 M MgCl$_2$ for 1 h at 37°. Electrophoresis was carried out in 5% acetic acid, adjusted to *p*H 3.5 with pyridine, on Whatman 3MM paper for 1.5 h at 4000 V and on DEAE-paper for 2.5 h at 3000 V. The labelled compounds were visualised by autoradiography. The identity of pUp in *a* and pU in *b* was verified by two-dimensional thin-layer chromatography.

almost twice as high as that found in ApGp after RNase A digestion of oligo II, although both products contain two phosphates (Table 1). The discrepancy can be explained in that the polio ^{32}P-RNA used in our study was not uniformly labelled. This was detected as follows. The base composition of the virion ^{32}P-RNA, when analysed by the isotope dilution method after complete digestion with RNase T2, is Cp = 24.3%, Ap = 30.1%, Gp = 22.5%, and Up = 23.1%, reflecting the base composition of unlabelled RNA[16]. A different base composition, however, is obtained after digestion of the ^{32}P-RNA with nuclease P1 or snake venom exonuclease which yield 5′-monophosphates (pC = 21.2%; pA = 30.5%; pG = 12.7%; pU = 35.6%). These observations suggest that the precursor pools for viral RNA synthesis were not fully equilibrated during growth of the virus. Instead, the specific radioactivity of ^{32}P-GTP and ^{32}P-CTP in the infected cell was lower than that of ^{32}P-UTP. As outlined in Table 1, we have corrected the observed radioactivity of oligonucleotides derived from the 5′ end of polio ^{32}P-RNA for the difference of the specific radioactivity of their individual monophosphates. The corrected values are close to those expected. Based on the cleavage products found and because Y and Y′ contain pUp, we conclude that the 5′ end of the poliovirus genome is homogeneous and has the sequence VPg-pU-U-A-A-A-A-C-A-Gp.

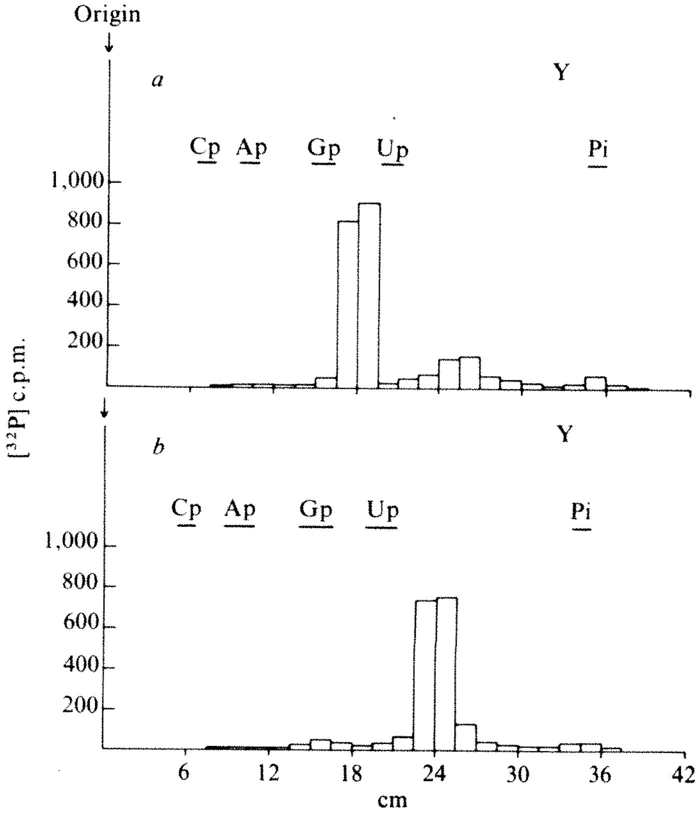

Fig. 3 2.5×10^3 c.p.m. each of ^{32}P-T2-Y (see Fig. 1*d*) were incubated for 2 h at 37 °C, *a*, in 10 μl 0.05 M sodium phosphate, *p*H 8, or *b*, in the same buffer with 0.5 units aminopeptidase M (Sigma). Samples were electrophoresed on Whatman 3 MM paper as described in Fig. 2. 1.5 cm strips of the paper were monitored for radioactivity.

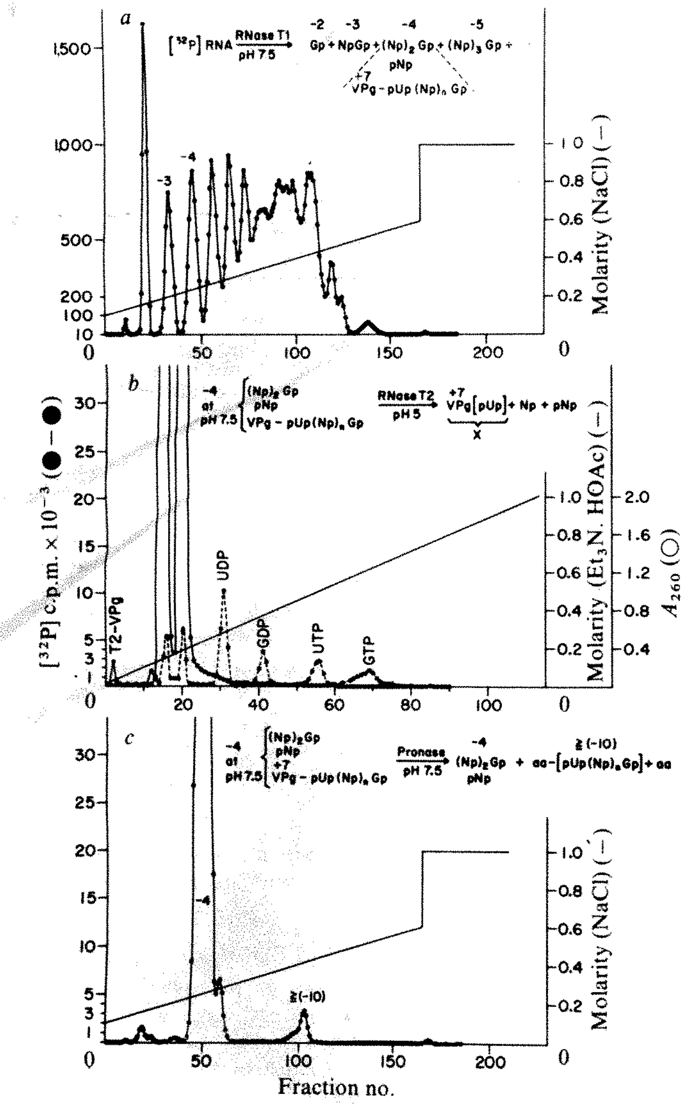

Fig. 4 Isolation of the 5′-terminal oligonucleotide of poliovirion RNA. *a*, 400 μg (4×10^8 c.p.m.) of poliovirion ^{32}P–RNA were digested with 50 units of RNase T1 (Calbiochem) in 200 μl 0.05 M Tris-HCl, *p*H 7.5, 0.002 M EDTA for 2 h at 37 °C. The mixture was applied to a DEAE–Sephadex A25 column and the components eluted with a linear gradient (200 ml) of 0.1–0.6 M NaCl in 0.02 M Tris-HCl, *p*H 7.5, 7 M urea. *b*, Peak −4 of Fig. 4*a* was desalted, digested with RNase T2 and chromatographed on a DEAE–cellulose column in triethylammonium acetate, *p*H 5, as described in Fig. 1*b*. X corresponds to the nucleotidyl-protein T2-VPg. *c*, Peak −4 of Fig. 4*a* was desalted, digested in 50 μl buffer with Pronase as described in Fig. 1*c*, and chromatographed on DEAE–Sephadex as described in Fig. 4*a*. Peak (≥ −10) was pooled and separated into two oligonucleotides ('oligo I' and 'oligo II') by two-dimensional gel electrophoresis (see text).

From the net charge of phosphates of the 5′-terminal oligonucleotidyl-protein (calculated to be −11) and its elution at peak −4 (Fig. 4*a*) we estimate that VPg must have a net positive charge of at least +7 at *p*H 7.5. The positive charge and the affinity to surfaces of polio VPg resemble properties of small nuclear proteins, for example histones of the host-cell. Available evidence suggests, however, that VPg is virus coded as it can be labelled with amino acids 3 h after infection when host protein synthesis is inhibited[11]. Moreover, we have identified a protein linked to genome RNA of another picornavirus, the encephalomyocarditis virus, which was grown in HeLa cells as was poliovirus. The polio-VPg and the EMC-VPg, although isolated from the same host cell, are slightly different in their molecular weights, an observation supporting our notion that the VPg's are virus specific proteins (Golini, A. N. and E. W. unpublished).

VPg is linked to nascent strands of polio RI and to minus strands

The polio replicative intermediate (RI) has been identified as the only structure involved in the synthesis of viral single stranded progeny RNA (see ref. 17 for review). We have, therefore, analysed RI for VPg since the presence of VPg might identify the step in viral replication during which the protein is attached to the viral RNA. ^{32}P–RI was isolated from polio virus-infected HeLa cells as previously described[18]. Absence of free plus strand RNA was shown by gel electrophoresis in 1% Agarose (data not shown; compare ref. 18). Digestion of denatured ^{32}P-RI with RNase T2 and separation of the products by column chromatography as in Fig. 1*b* revealed that the structure does not contain pppNp, ppNp or capping groups (data not shown). Some material eluting at the position of nucleoside diphosphates was analysed by paper electrophoresis and found to contain all 4 pNp's: we assume this material originated from suprious ends in the RI. Material eluting in the void volume was shown to be T2-VPg by (1) subsequent gel electrophoresis, in which it co-migrated with virion RNA-derived T2-VPg, and (2) by digestion with Pronase and snake venom exonuclease which yielded T2-Y and pUp, respectively (data not shown). The number of nascent

Fig. 5 Separation of digestion products by electrophoresis on DEAE-paper in pyridinium acetate, *p*H 3.5 (see Fig. 2) at 3,000 V for 4 h. Oligo I (*a*) and Oligo II (*b*) (see Fig. 4*c*), which had been purified by two-dimensional gel electrophoresis, were digested in 10 μl 0.05 M Tris-HCl, *p*H 7.5, 1 mM EDTA with RNase A for 1 h at 37 °C, or in 10 μl 0.05 M ammonium acetate, *p*H 4.5, 1 mM EDTA with 0.1 unit RNase T2 for 30 min at 37 °C. Oligo II (*b*) was digested in 10 μl acetate buffer, *p*H 4.5, with 0.05 units RNase U2 for 1 h at 37 °C. Radiolabelled compounds were visualised by autoradiography. The identity of oligonucleotides was verified by secondary digestions with RNase T2. Note that digestion with RNase U2 is incomplete in these conditions and yields 2′,3′-cyclic phosphates[15]. For quantitation of the products see Table 1.

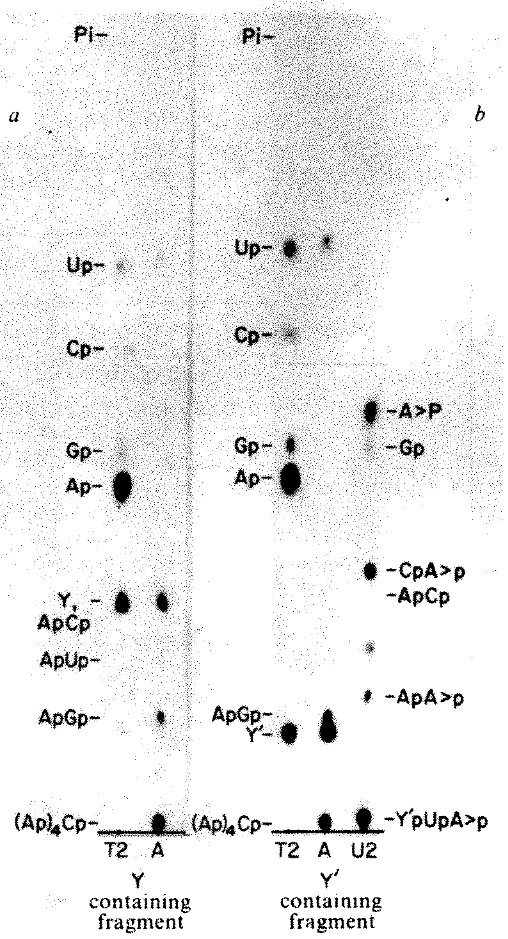

Table 1 Products of nuclease digestion of the polio 5′-terminal oligonucleotide

	RNase T2*			RNase A*		RNase U2*†
Products	Yield (c.p.m.)	Corrected‡	Products	Yield (c.p.m.)	Corrected‡	Products
Y′ (Z′ pUp)	1,566	1,018	A_4Cp	1,990	2,029	Y′–UA > p
Ap	1,957	2,310	Y′ (Z′ pUp)	996	647	AA > p
Gp	422	485§	AGp	450	657§	Unknown
Cp	534	528	Up	438	434	CA > p
Up	699	692				Gp
						A > p

*Digestion of oligo II and identification of products as described in Fig. 5.

†Digestion with RNase U2 is incomplete. Tle unknown spot was absent in other experiments.

‡The observed counts for each phosphate are corrected for the deviation from uniform labelling of the RNA by multiplying the values with a factor f (f = [% Np in total RNA after RNase T2 digestion]: [% pN in total RNA after nuclease P1 digestion]). For values of base compositions see text. Factors of each 5′-phosphate are: pC = 1.15; pA = 0.99; pG = 1.77; pU = 0.65. For example, the value observed for Y′ (Z′pUp) is corrected by multiplying it with 0.65 since both phosphates originate from pU.

§Assuming that the nearest 3′-neighbour is C. The values are slightly lower if A or U are the nearest neighbours.

strands in RI can be derived from the resistance of the structure to digestion with RNase A (ref. 17). Based on an RNase A resistance of ^{32}P-RI of 36.4%, we calculated that the structure must contain on the average seven nascent RNA strands or 5.5 full length equivalents (the template strand included). This corresponds to 42.3 × 10³ nucleotides assuming 7.7 × 10³ nucleotides per polio virion RNA molecule. 32 × 10⁶ c.p.m. of ^{32}P–RI yielded 12,774 c.p.m. of ^{32}P-T2–VPg. If corrected for the higher than average specific radioactivity of the two UTP-derived phosphates in VPg, these counts correspond to five T2–VPg molecules which is 63% of the theoretically expected value of eight VPg's. Since losses are encountered during the isolation procedures of the adhesive protein[10] we suggest that each nascent strand of polio RI is linked to VPg at the 5′ end.

Evidence for VPg on minus strand RNA was obtained as follows: ^{32}P-RI (ref. 18) or ^{32}P-RF (refs 19, 20) were denatured and annealed to excess unlabelled virion RNA. This removed most labelled plus strand RNA (including the nascent strands) from minus strand RNA[20]. The hybrid double strands were then purified by gel filtration and the 5′-terminal ^{32}P-poly (U) isolated by affinity chromatography after RNase T1 digestion[20]. The poly(U) thus obtained contained in a typical experiment 1 VPg, 45 Up and 1–4 mol of Gp, Cp and Ap. Presence of bases other than Up would be expected[20]. The low levels of Gp, Cp and Ap, on the other hand, clearly indicate absence of contaminating plus strand RNA. The yield of ^{32}P-VPg, however, is higher than expected since the average length of poly(U) has been reported to be 60–90 nucleotides[18–20]. We have, at present, no explanation for the high yield. ^{32}P-VPg was isolated from ^{32}P-poly(U) by column chromatography as described in Fig. 1*b*. It had the same mobility in polyacrylamide gels as virion RNA-derived VPg and yielded after digestion with Pronase and snake venom exonuclease T2-Y and pUp, respectively (data not shown). We conclude that polio minus RNA is linked at the 5′ end to a small protein. This explains our previous failure to transfer phosphate with polynucleotide kinase to the poly(U) of phosphatase treated polio minus RNA[20].

The absence of terminal triphosphates and the presence of VPg on nascent strands of polio RI is direct evidence not previously reported that synthesis of poliovirus RNA occurs from 5′ to 3′. It also suggests that VPg may be involved in an early step of RNA synthesis.

Possible role of VPg in replication

Our previous failure to find pppNp in digests of polio RI suggested to us that initiation does not occur *de novo* but by an alternative mechanism, possibly by a primed reaction (see discussion in ref. 5). The5 ′-terminal structure of polio virion RNA, of nascent strands in polio RI, and of polio minus strand RNA, which we report here, implicates a most unusual molecule for the role of a primer in RNA synthesis: a small, basic protein. Our current view of the role of that protein, VPg, in replication

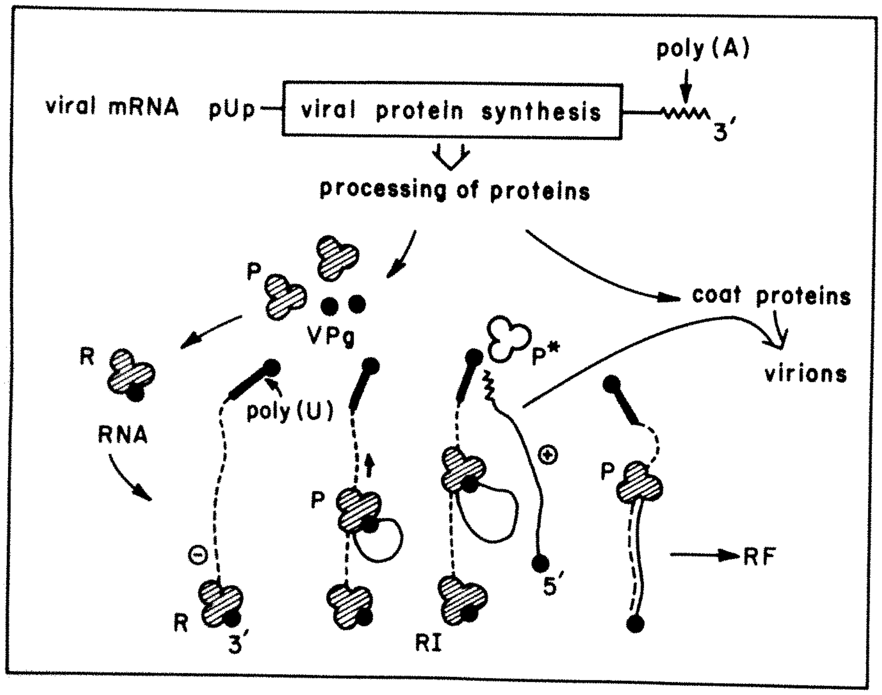

Fig. 6 Involvement of the genome-linked protein VPg in polio virus RNA synthesis. The model shows only synthesis of plus strands but applies also to synthesis of minus strands. RI, replicative intermediate; RF, replicative form (double stranded RNA); R, replicase; P, polymerase.

is illustrated in Fig. 6. Following the post-translational cleavage of viral proteins (see ref. 21 for review), newly made VPg associates with newly made protein P (a hypothetical, virus-specific RNA polymerase) to form the replicase R. Replicase R, but not polymerase P, is able to initiate plus or minus strand synthesis whereby VPg is linked to the nascent RNA. Strand elongation will then proceed during which the RNA-linked VPg remains attached to the polymerase. This condition would prevent the nascent strand from annealing to the complementary template RNA. After the transcription of RNA and its 3′-terminal poly(A) (ref. 22) has been completed, progeny strands and polymerase P* are released. The released polymerase P* may or may not be competent to reassociate with free VPg and re-enter the replicative cycle. It should be stressed that VPg could enter the replication complex also in form of the peptide chain of a precursor protein. Initiation of RNA synthesis may then occur concomitantly with proteolytic cleavage of the precursor yielding a nucleotidyl-protein.

Should the RNA-linked VPg dissociate from polymerase P after initiation of RNA synthesis, strand elongation will occur in the absence of the 'initiator' protein. As a consequence the nascent strand may anneal to the template RNA thus generating double-stranded RNA, the polio RF. In fact kinetic studies[17] and biochemical studies[19] have shown that polio RF is a by-product of replication and not an artefact of isolation procedures.

A tight coupling of poliovirus RNA synthesis to viral protein synthesis exists throughout the infectious cycle even though the sites of translation and replication are physically separated[17]. Our model offers an explanation for this control mechanism: the attachment of protein VPg to RNA requires that VPg be continuously supplied by the translational machinery.

Finally, one striking finding is that the viral mRNA is not identical to virion RNA as previously assumed[17] in that it lacks VPg. Instead, polio mRNA is 5′-terminated with pUp[5-7]. This requires that VPg be cleaved from infecting virion RNA. To what extent this type of 'processing' occurs—cleavage of just the protein or of an oligonucleotidyl-protein—will be known when we have identified the 5′-terminal nucleotide sequence of viral mRNA. Removal of the positively charged protein could be obligatory for translation and might, at the same time, render viral mRNA incompetent for encapsidation. In fact, mRNA is not a precursor for virions[17] which might implicate VPg in the morphogenesis of the virion. It should be mentioned that the protein is not essential for virion RNA to be infectious since RNA from which VPg has been removed proteolytically fully retains its specific infectivity (Golini, A. N. and E. W., unpublished).

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Purification and structural characterisation of human *HLA*-linked B-cell antigens

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The human B cell-specific alloantigen which is closely linked genetically to HLA *contains two non-covalently associated, sialogycoprotein subunits of molecular weight (MW) 29,000 (p29), and 34,000 (p34). Although p29 and p34 have different amino-terminal sequences, their tyrosine peptide maps indicate considerable similarity in other portions of their polypeptide chains. Thus the genes for their proteins may have evolved by duplication of a common ancestral gene. Another lymphocyte cell surface protein of MW 16,000 (p16) has also been characterised. Both p16 and p44 (the heavy chain of HLA-A,B antigens) have been compared with p29 and p34.*

A HUMAN system of cell surface alloantigens has recently been identified which is expressed predominantly on B lymphocytes, and is determined by a gene in *HLA* (refs 1–4), the major histocompatibility complex (MHC) of humans. At least two genetic loci for B-cell alloantigens have been identified in the *HLA* region separable by crossing over[5]. One of these loci is either identical to or in very strong linkage disequilibrium with the *HLA*-D locus, the genetic locus causing the strongest stimulation of the mixed lymphocyte culture (MLC) (ref. 1). Human *HLA*-linked B-cell antigens are serologically distinct from the cell surface antigens which are products of the classical *HLA*-A and *HLA*-B loci. It has previously been shown that papain solubilised and purified *HLA*-linked B-cell alloantigens consist of a complex of two polypeptides of 23,000 (p23) and 30,000 (p30) molecular weight (MW) (refs 6–8). Antisera raised against this complex are potent inhibitors of the MLC reaction and precipitate polypeptides of 29,000 and 34,000 MW (refs 6–9) from detergent-solubilised extracts.

This report describes the isolation of detergent soluble *HLA*-linked B-cell alloantigens, analysis of their subunit structure, and characterisation of the subunits by amino acid analysis, NH_2-terminal sequencing, and peptide mapping.

Purification

Membranes were prepared from RPMI 4265 lymphoblastoid cells, solubilised with the non-ionic detergent Brij 99 : Brij 97 (2 : 1), and after high speed centrifugation, the detergent soluble supernatant was adsorbed on to and eluted from a *Lens culinaris* lectin affinity column[10,11]. The eluate was subjected to gel filtration on a BioGel A-5m column (Fig. 1). Fractions from the area of the column containing protein were analysed by sodium dodecyl suphate (SDS) gel electrophoresis (Fig. 1, inset) and placed in pools A, B and C. The material applied to the BioGel A-5m column consisted predominantly of six polypeptides of MW 105,000 (p105), 44,000 (p44), 34,000 (p34), 29,000 (p29), 16,000 (p16), and 12,000 (p12). p105 and p16 were in a sharp peak at 900,000 MW (pool A), and p44 and p12, the subunits of HLA-A,B antigens, were in a sharp peak at 400,000 MW (pool C) with some aggregated p44,12 at higher MW. p29 and p34, which are the subunits of the *HLA*-linked B cell alloantigen (see below), chromatographed at an intermediate MW in a broad peak indicative of partial aggregation (pools A and B) and were largely separated from p44,12. The fact that the ratio of p29 to p34 was constant in all fractions of the column was consistent with non-covalent association between p29 and p34. Membrane suspensions were prepared in the presence or absence of 1 mM dithiothreitol (DDT) (refs 13, 14). The preparations with no DDT were somewhat less pure, but p29 and p34 showed a similar elution pattern during BioGel A-5m filtration.

Subunit structure

After preparation in the absence of DDT, the fractions from BioGel A-5m filtration enriched in p29 and p34 were incubated with 0.6% SDS at various temperatures, in the presence of iodoacetamide (IAA) or 2-mercaptoethanol (ME) and electrophoresed on SDS gels (Fig. 2*a*, *b*). Comparison of the patterns at different temperatures showed that the HLA-A,B antigen subunits, p44 and p12, were dissociated and electrophoresed identically after incubation at all temperatures. But, after incubation at 21, 27 or 38 °C in SDS, p29 and p34 were associated in a complex (p29,34) at an apparent MW of 55,000, but were dissociated at 100 °C. Interestingly, when pooled human IgG was similarly treated with ME and SDS at 21 °C, L chains were dissociated, but H chains remained associated non-covalently (at the ME concentration used[15]) in an H_2 dimer which calibrated at 90,000 MW (Fig. 2*d*). Since dissociation into p29 and p34 occurred at 100 °C in the presence of 10 mM iodoacetamide to prevent disulphide interchange (Fig. 2*a*), it may be concluded that p29 and p34 are not linked by disulphide bonds. The mobilities of the subunits in reduced gels (Fig. 2*b*) were different from those in unreduced gels (Fig. 2*a*), particularly in the case of p29. The identity of reduced and unreduced bands was confirmed by cutting out bands from unreduced gels, reducing and electrophoresing them. Two dimensional SDS gels of the same preparation using electrophoresis without reduction in the first dimension, followed by reduction and electrophoresis in the second dimension, also showed that while p44 disulphide-linked dimers were present in these preparations, p29 and p34 were not disulphide-linked (see Fig. 7 in ref. 13 and Fig. 2 in ref. 14). But, cross-linking experiments with two cleavable cross-linking reagents containing S–S bonds[16,17] and isoelectric focusing showed that p29 and p34 are non-covalently associated with each other and that dimers of p29 alone or of p34 alone are not present (see Figs 3 and 4 in ref. 18).

Purified p29,34 was prepared by incubation of pool B in SDS at room temperature, followed by preparative SDS gel electrophoresis. Immunisation of rabbits with this material elicited a B cell specific antiserum with properties much like the B cell specific anti-p23,30 heteroantisera reported previously (refs 6–8, 18; T.A.S., J.F.K. and A. Fuks, in preparation). Moreover, purified p29,34 antigen specifically inhibited an *HLA*-linked B cell alloantiserum Po[41]. Heat-dissociated p29 and p34 did not elicit a detectable antibody response in rabbits and did not inhibit either the human alloantiserum or the rabbit heteroantiserum prepared against the p29,34 complex.

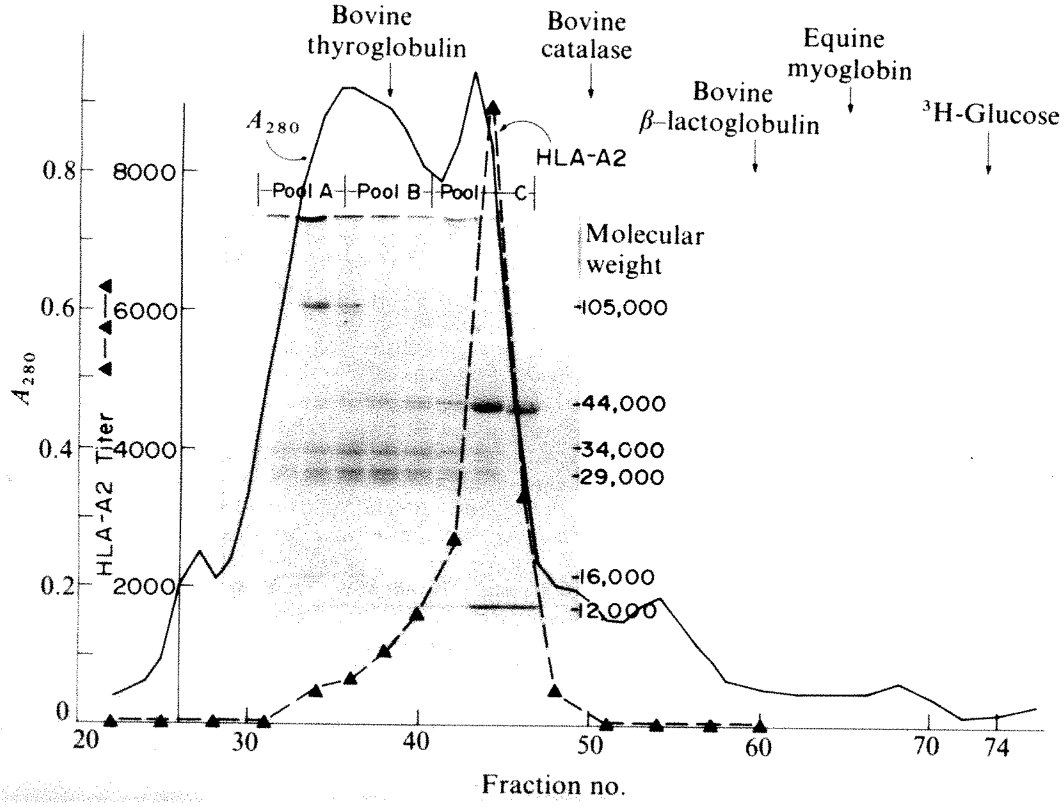

Fig. 1 BioGel A-5m filtration and SDS gel electrophoresis profile of p29,34. BioGel A-5m filtration of the lectin column eluate was as described[10,11]. Equal volumes of the even numbered fractions 30–48 were precipitated with five volumes of acetone and electrophoresed on Laemmli 11% polyacrylamide–SDS slab gels[12], after boiling in 5% ME sample buffer, and in the figure are aligned with their respective BioGel fractions.

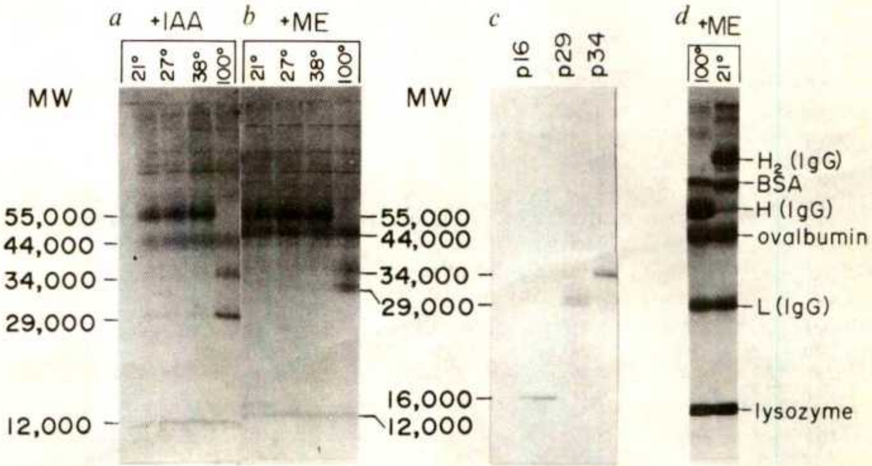

Fig. 2 SDS gels of p29,34 preparations after various treatments. *a*, SDS gel electrophoresis in 10% acrylamide Laemmli gels[12] of material purified in the absence of DTT by lectin affinity chromatography and gel filtration[13,14] after incubation with 0.6% SDS and 10 mM IAA for ½ h at 20, 27 and 38 °C or for 5 min at 100 °C. *b*, As above, except incubation was in the presence of 5% ME instead of IAA. *c*, 2 μg of each polypeptide eluted from gels[19] was electrophoresed in an SDS7–15% acrylamide gradient gel. *d*, A mixture of standards (IgG, bovine serum albumin (BSA), ovalbumin, and lysozyme) were electrophoresed after treatment as in (*b*).

Chemical characterisation

The subunits of the *HLA*-linked B-cell alloantigen, p29 and p34, as well as p16, an unrelated protein which also bound to *Lens culinaris* lectin affinity columns and was obtained in good yield, were chemically characterised.

Materials in pools A and B were combined, alkylated with 3 mM iodoacetic acid and heated at 100 °C in the presence of SDS. Proteins were then purified by preparative electrophoresis in 0.8×22 cm, 10% polyacrylamide tube gels using precautions described by Weiner *et al.*[19]. Advantage was taken of the greater separation of p29 and p34 in the unreduced state (see Fig. 2*a*). After elution, the proteins were chromatographed twice on a 0.9×40-cm Sephadex G-50 column equilibrated with 20 mM NH_4HCO_3 to remove SDS and substances which interfered with the Edman degradation, and were lyophilised for amino acid and NH_2-terminal sequence analysis. Equimolar amounts of p29 and p34 were recovered, as measured by either the Lowry assay[20] or amino acid analysis. Analytical electrophoresis of the eluted polypeptides (Fig. 2*c*) showed them to be homogeneous.

The peptides were also shown to be homogeneous by amino-terminal analysis using the SDS–dansyl technique[19] and by automatic amino-terminal sequencing (Table 1). The amino-terminal sequences of p29 and p34 are quite different from one another, and from p16. Strikingly, the NH_2-terminal amino acids of p29 and p34 are identical to those of p44 and p12 (refs 11, 21, 22) although no further homologies are obvious. Much more extensive sequence data for all of these polypeptides will be required to examine the possibility of homology among them.

The amino acid compositions of p16, p29 and p34 were determined by performing hydrolysis for 24, 48 and 72 h and applying samples at two concentrations to a Beckman 121 M analyser equipped with a microbore single column system (Table 2). The composition of p44 from detergent soluble HLA-A,B antigens (a mixture of three specificities)[10,11] is included in Table 2 for comparison. The amino acid compositions of p29 and p34 are strikingly similar, suggesting that these proteins may be related. The amino acid compositions showed that p29 and p34 do not contain an unusually low percentage of polar residues, suggesting that only a short segment of their polypeptides is involved in membrane binding. This is consistent with the finding that papain-solubilised *HLA*-linked B cell alloantigens (p23,30) contain polypeptides of only slightly shortened length[6–8].

The sialic acid content (Table 2) was 2.2 and 2.6 mol per mol for p29 and p34 respectively, and thus they are both sialoglycoproteins with amounts of sialic acid similar to that of HLA-A,B antigen heavy chains[21]. Staining of SDS gels with periodic acid-Schiff reagent (PAS) also showed that p29 and p34 are glycoproteins. Since glycan moieties which have affinity for the *Lens culinaris* lectin are generally linked by a glycosylamine bond to asparagine and are branched oligosaccharides with galactosyl-*N*-acetylglucosamine sequences in the outer chains and mannose residues in the core[26], the carbohydrate compositions of p29 and p34 may be similar to that of p44 (ref. 21). p16 contained 0.5 mole sialic acid per mol, but PAS staining was not detected.

Since p29 and p34 are glycoproteins, their true MWs may be lower than the MW estimated by SDS gel electrophoresis, as is the case for the heavy chain of HLA-A,B antigens[21]. Using these molecular weights as a starting point, the peptide MWs of p29, p34 and p16 were calculated from the amino acid compositions[27] and found to be 23,600; 28,400; and 15,600 respectively, and the latter MWs were used for calculation of amino acid composition in residues per molecule (Table 2). Three cysteines were found in p29 and p34 (although cysteine may have been underestimated since p12 contained 0.8 residue per mol). p29 and p34 which had been treated with 1 mM DTT and 3 mM iodoacetic acid in the absence of SDS in order to alkylate easily reduced S–S bridges and free SH groups, were also analysed. They contained 0.8 and 1.0 residue of

Table 1 Amino terminal sequences

	Residue														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
p16	Thr	Glu	Thr	[Thr]		Phe				Lys	Phe		Pro	Asp	
p29	Gly	Asp	Thr	Pro			Phe	Leu	Glu	Gln	Val				
p34	Ile	Lys	Glu	Glu	[Arg]	Val	Ile		Gln	Ala	Glu	Phe	Tyr	Leu	Asn

Peptides (10–30 nmol) were sequenced using ^{35}S-phenylisothiocyanate (New England Nuclear) in an updated Beckman 890 B Sequencer[21]. Phenylthiohydantoins were identified after thin-layer chromatography on polyamide sheets by visual inspection and autoradiography. Arginine, lysine, isoleucine and leucine were identified on the amino acid analyser after back hydrolysis. Peptides were also sequenced using the SDS–Edman–dansyl technique[19].

Table 2 Amino acid and sialic acid composition

Amino acid	mol per 100 mol p16	mol per 100 mol p29	mol per 100 mol p34	mol per 100 mol p44**	residues per molecule p16	residues per molecule p29	residues per molecule p34
CM-Cys	0.55	1.50	1.21	1.31	0.8(1)	3.1(3)	3.0(3)
Asp	12.14	7.87	8.93	8.73	17.0(17)	16.2(16)	22.3(22)
Thr*	10.78	6.70	6.65	6.87	15.1(15)	13.8(14)	16.6(17)
Ser*	7.32	6.87	5.32	6.77	10.2(10	14.2(14)	13.2(13)
Glu	7.80	12.62	11.61	12.65	10.9(11)	26.0(26)	29.0(29)
Pro	4.37	4.82	6.26	5.06	6.1(6)	9.9(10)	15.6(16)
Gly	7.04	7.93	6.89	7.6 7	9.9(10)	16.4(16)	17.2(17)
Ala	7.16	4.85	5.27	8.32	10.0(10)	10.0(10)	13.1(13)
Val†	7.90	8.14	8.48	6.19	11.1(11)	16.8(17)	21.1(21)
Met	0.63	1.52	1.46	1.51	0.9(1)	3.1(3)	3.6(4)
Ile†	5.31	3.33	4.89	3.45	7.4(7)	6.9(7)	12.2(12)
Leu	5.94	8.56	9.17	6.94	8.3(8)	17.7(18)	22.9(23)
Tyr	3.58	3.76	2.73	4.32	5.0(5)	7.8(8)	6.8(7)
Phe	6.21	5.31	5.98	3.06	8.7(9)	10.9(11)	14.9(15)
His	1.34	2.83	2.70	2.91	1.9(2)	5.8(6)	6.7(7)
Lys	5.79	3.88	4.45	3.87	8.1(8)	8.0(8)	11.1(11)
Arg	3.24	7.01	5.56	7.19	4.6(5)	14.5(14)	13.8(14)
Trp‡	2.90	2.51	2.44	(3.2)¶	4.1(4)	5.2(5)	6.1(6)
Polarity §	48.4	47.8	45.3	49.0			
Sialic acid ‖					0.5	2.2	2.6

Values in parentheses rounded to the nearest integer.
* Extrapolated to zero time values.
† 72-h values.
‡ Determined by using a modification of the method of Edelhoch (23) in 0.01% SDS.
§ Calculated according to ref. 24.
‖ Determined by the method of Warren (25).
¶ Assumed to be the same as for the papain product (21).
**From refs 10 and 11.

carboxymethyl-cysteine per mol respectively. The additional cysteines of p29 and p34 were either in intrachain disulphide bridges or unreactive. The fact that the mobilities of p29 and p34 in SDS gels (Fig. 2*a*, *b*) are slower after reduction suggests that they each contain at least one intrachain disulphide bridge. Even during purification in the absence of DDT, as shown above the easily alkylated cysteines are not oxidised to form interchain disulphide bridges between p29 and p34.

Subunit homologies in tryptic peptide maps

Although limited NH_2-terminal sequencing did not reveal homology between p29, p34 and p44, homologies between polypeptides of dissimilar size do not necessarily begin at the NH_2-terminus. Since the amino acid compositions suggested such an homology, these proteins were compared by tyrosyl peptide mapping.

Carboxymethylated p16, p29, p34 and p44 were heated at 100 °C in the presence of SDS and labelled with ^{125}I. After removal of SDS, they were digested with trypsin[28]. Comparison of the ^{125}I-tyrosine tryptic peptides by one-dimensional silica-gel chromatography (Fig. 3*a*) showed that p29 and p34 had an almost identical peptide pattern. Spots due to free iodine, present in all samples, migrated close to the solvent front in the one dimensional system (and moved out of the area shown on the two dimensional maps illustrated in Fig. 3*b*). Two-dimensional maps (Fig. 3*b*) showed that p29 and p34 shared six major ^{125}I-tyrosine tryptic peptides (labelled A and B). p29 and p34 also each seem to contain unique peptides (labelled C and D) which agrees well with amino acid analysis showing p29 and p34 to contain eight and seven tyrosines, respectively. p44 showed considerable differences but seemed to contain one peptide (A) in the same position as a peptide of both p29 and p34. No significance should be attached to this single similarity without much further study. p16 showed no homology with

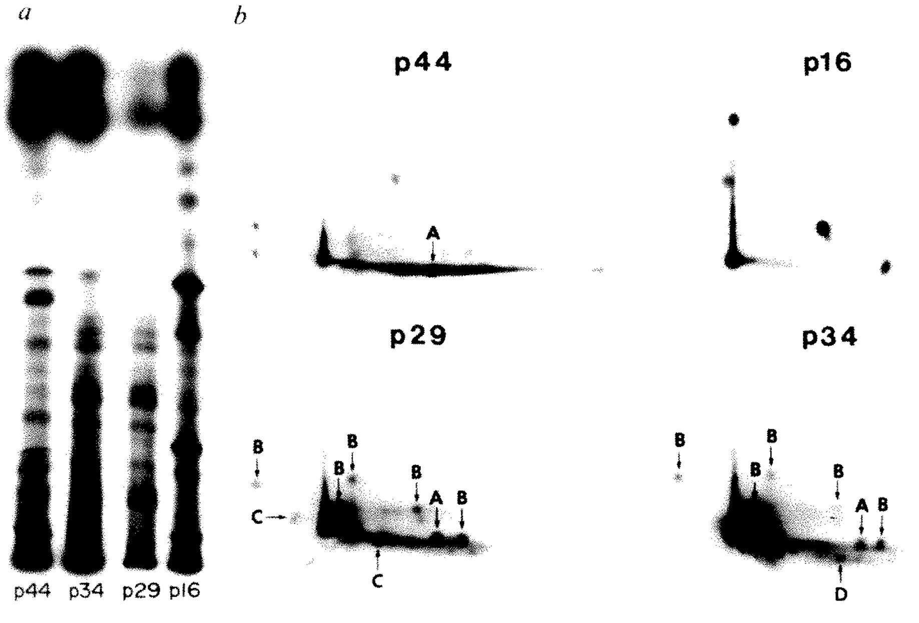

Fig. 3 ^{125}I-tyrosine-labelled tryptic peptide maps of p16, p29, p34 and p44. Peptides were labelled with ^{125}I in the presence of SDS, digested with trypsin, and separated on silica gel G (ref. 28). HLA antigens were prepared as described in refs. 10 and 11 and p44 was separated from p12 by preparative SDS gel electrophoresis[19]. *a*, Chromatography dimension only. *b*, Chromatography (upward) followed by electrophoresis (anode to right). The origin is the intensely radioactive spot near the lower left corner. Labelled spots: A, peptides common to p29, p34 and p44; B, peptides common to p29 and p34; C, peptides unique to p29; D, peptide unique to p34.

any of the other peptides and provided evidence that none of the homologies observed in two-dimensional maps were due to artefacts. Thus p29 and p34 are highly homologous to one another. p29 and p34 could not be proteolytic products of p44, since four peptides not present in p44 were found in p29 and p34. Several pieces of evidence show that p29 is not a proteolytic breakdown product of p34. p29 contained one more serine and tyrosine than p34 (Table 2) and two unique tyrosine tryptic peptides (Fig. 3). Additionally, p34 contained a tyrosine residue at position 13 (Table 1), and therefore, proteolytic cleavage from the NH_2-terminus would have produced a product containing two fewer tyrosines than actually found in p29. Proteolysis of p29 and p34 during preparation or storage was never noted, and the ratio of the two polypeptides always remained constant, as determined by SDS gel electrophoresis. Equimolar amounts of p29 and p34 were recovered after preparative SDS gel electrophoresis. Proteolysis would have been expected to result in a mixture of molecules of $(p34)_2$, $(p29)_2$ and $(p29)_1(p34)_1$ structures, but only molecules of the latter structure were found in cross-linking experiments. p29 and p34 each also seemed to be uniquely cleaved by papain digestion, since one polypeptide was converted to p23 and the other to p30. Recent studies of somatic hybrids have also suggested that p29 and p34 are products of different genes[29].

Two likely explanations for the high degree of homology between p29 and p34 remain. A common portion could be encoded by one gene, and unique portions, probably at the NH_2-terminus could be encoded by different genes (a two gene, one polypeptide model, as in antibodies). Alternatively, the genes for p29 and p34 could have arisen by duplication of a common ancestral gene. Since this is the case for two other genes in *HLA*, *HLA-A* and *HLA-B* (ref. 21), this seems a likely explanation. Inhibition of the alloantiserum suggests that at least one of the p29 and p34 subunits is a product of one of the *HLA* gene loci which code for B cell alloantigens. But, since genes which code for homologous subunits are often unlinked (as is the case for the immunoglobulin L and H chain genes[30]), p29 and p34 would not necessarily both be products of the *HLA* region.

If the tyrosine tryptic peptides are representative of the remaining three-quarters of the tryptic peptides of p29 and p34, these molecules would be identical throughout most of their sequences. It will be interesting to determine by further structural analysis whether non-covalent association occurs between homologous regions, and whether differences between the subunits are confined to the NH_2-terminal region. Since RPMI 4265 cells may be heterozygous, and in analogy to the mouse *I* subregions[31], closely linked loci might specify chemically similar yet distinct molecules, p29 and p34 preparations may well contain chemically different populations of molecules, which might explain the electrophoresis of p29 as a doublet in high resolution Laemmli SDS gels (Fig. 1). Such differences must be limited, however, as no obvious heterogeneity was detected by NH_2-terminal sequencing and close to the expected number of major tyrosine tryptic peptides was obtained.

Relationship to murine and guinea pig Ia antigens

Genetic and serological studies have demonstrated the following similarities between murine Ia antigens and human *HLA*-linked B-cell alloantigens. (1) There genes are in the MHC and in intimate association with MLC genes[1,31]; (2) Antisera to these products are potent inhibitors of the MLC reaction[6,7,31] and also block generation of plaque-forming cells *in vitro*[32]. And (3), they have similar tissue distributions[1-9,31]. Our report shows that the two-chain, glycoprotein structure of *HLA*-linked B-cell alloantigens seems similar to that of murine and guinea pig Ia antigens[28,31,33]. Moreover, the NH_2-terminal sequence of the guinea pig Ia antigen smaller chain[33] is identical to that of human p29 in three out of seven positions, strongly suggesting inter-species homology. Similarly, sequence studies have shown that *HLA-A* and *HLA-B* antigens are homologous to murine H-2 D and K antigens[34] and to guinea pig GP-LA B antigens[33]. However, murine and guinea-pig Ia antigens analysed after immunoprecipitation have been found on some but not other occasions to contain a disulphide bridge between their subunits[33,35]. Thus, these rodent Ia antigens might also contain free cysteines in each subunit, but would differ from the human analogues studied here in that their sulphydryls can readily form an interchain disulphide link.

It is not yet known whether *HLA*-linked B cell alloantigens resemble murine and guinea pig antigens in a further respect. The latter alloantigens are termed immune associated (Ia) because their genes co-map with (but are not necessarily identical to) immune response (*Ir*) genes[31]. This type of close association has not been established in humans. However, susceptibility to certain diseases, which may reflect the absence of particular *Ir* genes, is linked to the *HLA* region[36].

Ir gene function may be expressed in T cells, B cells, or both[37-39]. Stimulated T cells express Ia antigens, although in lower amounts than B cells[39,40]. It is thus possible, although not yet demonstrated, that Ia antigens may in fact be the products of *Ir* genes. If so, the homologies between the subunits of *HLA*-linked B-cell alloantigens would have implications for its role in the immune system as a human *Ir* gene product. Proteins with homologous subunits (immunoglobulins, haemoglobins, and isozymes are examples) often can be composed of any of a set of interchangeable subunits which are specified by duplicated genes. Since many unique combinations can be made using a much smaller number of unique subunits, great diversity in functional specificity can be generated in an economical fashion.

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letters to nature

Water vapour concentrations at the mesopause

MEASUREMENTS of atmospheric water vapour concentrations have been confined to altitudes below about 50 km showing an average volume mixing ratio of about 4×10^{-6} in the middle and upper stratosphere[1]. Only limited and rather indirect observational information on water vapour exists above 50 km including, for example, the presence of hydrated positive and negative ions and noctilucent clouds likely being composed of water ice particles. In a previous attempt to derive water vapour concentrations from measurements of hydrated positive ions, a mixing ratio between 1 and 2×10^{-6} below 78 km and a sharp decrease above was inferred[2]. In this paper, water vapour concentrations are derived at heights between 85 km and 94 km from two rocket-borne mass spectrometer measurements of atmospheric positive ions. The experiments took place at Andoya (69°N, 16°E) on May 31 1972 (ref. 3) and at Kiruna (68°N, 21°E) on August 18 1974 (ref. 4).

The method applied is based on the atmospheric ion reactions

$$NO^+\cdot CO_2 + H_2O \longrightarrow NO^+\cdot H_2O + CO_2 \tag{1}$$

$$O_2{}^+\cdot H_2O + H_2O \longrightarrow H_3O^+\cdot OH + O_2 \tag{2}$$

$$H_3O^+\cdot OH + H_2O \longrightarrow H_3O^+\cdot H_2O + OH \tag{3}$$

The reaction coefficients $k_2 = 1.9\times10^{-9}\ cm^3\ s^{-1}$ (ref. 5) and $k_3 = 3.2\times10^{-9}\ cm^3\ s^{-1}$ (ref. 6) are known from laboratory measurements. They are expected to have no significant temperature dependence. Although no laboratory value exists for k_1, it is reasonable to adopt a value of $1.5\times10^{-9}\ cm^3\ s^{-1}$, as most switching reactions have rate coefficients between 1 and $2\times10^{-9}\ cm^3\ s^{-1}$ (ref. 7). In the conditions considered (high electron densities in excess of $10^5\ cm^{-3}$) the dominating loss process for $NO^+\cdot H_2O$ and $H_3O^+\cdot H_2O$ was electron recombination. The recombination coefficient for $H_3O^+\cdot H_2O$ is $\alpha_2 = 3\times10^{-6}(300/T)^{0.5}\ cm^3\ s^{-1}$, (ref. 8) whereas for $NO^+\cdot H_2O$ an $\alpha_1 = 2\times10^{-6}\ (300/T)^{0.5}\ cm^3\ s^{-1}$ is adopted according to a systematics of α values[9]. Other loss processes for $NO^+\cdot H_2O$ and $H_3O^+\cdot H_2O$ can be neglected, as no ions other than NO^+ and $O_2{}^+$ were found in significant abundances besides $NO^+\cdot CO_2$, $NO^+\cdot H_2O$, and $H_3O^+.\ H_2O$. Processes decomposing $NO^+\cdot H_2O$ to form NO^+, like photodestruction[10] or thermal decomposition, are much slower than electron recombination. Instrumental effects like electric field or shock-induced collisional decomposition of the ions considered, can also be neglected as in these experiments cluster ions of much lower bond energies were measured between 85 km and stratospheric altitudes.

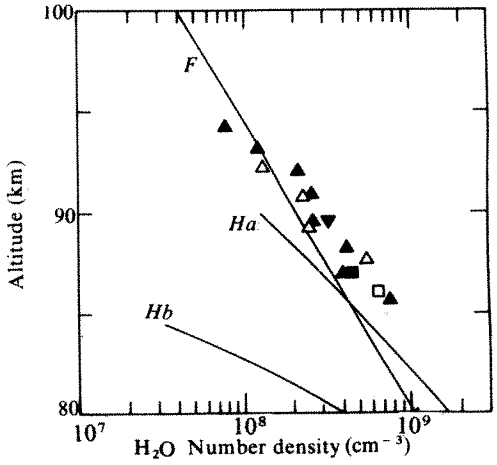
Assuming chemical equilibrium the continuity equations for $NO^+\cdot H_2O$ and $H_2O^+\cdot OH$

$$[NO^+\cdot CO_2]\,k_1\,[H_2O] = [NO^+\cdot H_2O]\,\alpha_1\,[e] \tag{4}$$
$$[O_2{}^+\cdot H_2O]\,k_2\,[H_2O] = [H_3O^+\cdot H_2O]\,\alpha_2\,[e] \tag{5}$$

relate the water vapour number densities to the measured ion densities, the measured electron density, and the reaction and recombination coefficients. Equation (5) neglects electron recombination of the intermediate ion $H_3O^+\cdot OH$ as reaction (3) is much faster. The electron density was measured by wave propagation as well as electrostatic probe experiments[11] with an accuracy better than ±20%. The error of the derived H_2O number densities is mainly introduced by the uncertainty of k_1 and k_2 and by the uncertain temperature dependence of α_1 and α_2. Relative H_2O-densities are not affected by changes in k_1 and k_2 and are only little affected by changes in the temperature dependence of α_1 and α_2 even if a maximum temperature dependence of $T^{-1.5}$ [12] is considered. The latter is true because height variations of atmospheric temperatures in the height range considered are relatively small. Regarding absolute H_2O-densities, an increase by as much as a factor of 2.6 has to be considered if a maximum temperature dependence of α_1, and α_2 of $T^{-1.5}$ is allowed for.

The water vapour number densities derived by use of equations (4) and (5) assuming atmospheric temperatures of the CIRA 1972 model atmospheres are shown in Fig. 1. For each flight both methods gave consistent densities. When compared with each other the H_2O density profiles of both flights agree closely decreasing from $8\times10^8\ cm^{-3}$ at 85 km to $8\times10^7\ cm^{-3}$ at 94 km. The experimental densities are slightly higher than those predicted by photochemical models including strong vertical eddy diffusion (*Ha*[13] and *F*[14]), but considerably higher than theoretical values based on weak vertical diffusion (*Hb*[13]). Using the measured H_2O densities and total gas densities from the CIRA 1972 (ref. 15) model atmosphere for 70° latitude in June and August volume mixing ratios were derived (Fig. 2). They range from 3.5 to 6×10^{-6} for the first and from 3 to 4×10^{-6} for the second experiment. The corresponding average values are 4.5×10^{-6} and 3.5×10^{-6} with no indication for a significant change in the height range 85 km to 94 km. These

Fig. 1 Number densities of water vapour for the experiments F33 (■●) and F36 (□ △) derived by the use of equations (4) (▲ △) and (5) (■ □). Theoretical profiles considering strong (*Ha*[13], *F*[4]) and weak vertical turbulent transport are shown for comparison.



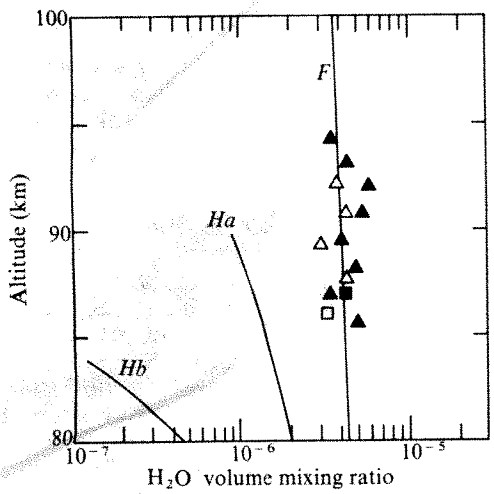


Fig. 2 Water vapour volume mixing ratios (for clarification Fig. 1 caption).

mixing ratios are compatible with the average value of 4×10^{-6} measured in the middle and upper stratosphere[1]. They are also close to a model profile considering strong vertical turbulent transport[14]. It may, therefore, be concluded that strong vertical transport must have been operative when our measurements were made. The transport mechanism may have been either strong turbulent diffusion or a mean upward motion which has been proposed to exist during summer at high latitudes[16]. Further implications from the high H_2O mixing ratios are enhanced hydration of atmospheric ions and enhanced water vapour condensation around nucleation embryos which may be pre-existing solid particles or complex ions. Concerning the ion induced nucleation, a high H_2O mixing ratio is of particular importance as it favours not only the growth of supercritical particles but also the formation of nucleation embryos by subsequent hydration of ions.

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Tectonic rotation in Western Canada

THE Queen Charlotte islands of British Columbia form the central part of the Insular Fold Belt, which is the most westerly tectonic unit of the North American Cordillera. In 1971, we collected oriented cores from the islands in an attempt to determine palaeomagnetically their motions relative to the stable craton of North America. A knowledge of these motions is needed before one can understand the origin of the Insular Fold Belt and the Cordillera generally. The localities sampled are difficult to reach, and only a reconnaissance was possible, but these preliminary results indicate that the islands have rotated clockwise since the Eocene.

The oldest rock unit of the Queen Charlotte Islands is the Late Triassic (Karnian) dominantly volcanic Karmutsen Formation[1]. It is overlain by the largely sedimentary Vancouver Group, which is cut by syntectonic foliated batholiths, the Older Plutons. The Older Plutons have K–Ar hornblende ages ranging from 156 to 142 Myr (refs 2–4). The Vancouver Group is unconformably overlain by the dominantly sedimentary rocks of the Long Arm Formation and the Queen Charlotte Island Group. Lying unconformably above these are the volcanics of the Masset Formation which have yielded a K–Ar mica age of 62±Myr (ref. 1) indicating a Palaeocene age. The Masset Formation is cut by the post-tectonic Younger Plutons. The Younger Plutons are massive unfoliated bodies, which have yielded K–Ar biotite and hornblende ages ranging from 39 to 26 Myr (ref. 1). This indicates that they are Oligocene, but the ages may represent the time of uplift and the bodies could have been emplaced in the Palaeocene or Eocene. The Masset is overlain unconformably by Neogene sediments. Samples were obtained from the Karmutsen Formation, the Older and Younger Plutons, and the Masset Formation (Fig 1). No coherent magnetisation was found in the Older Plutons (CP1, 2, 3, 14, 15 and 16, Fig. 1).

The Karmutsen Formation is over 4,000 m in thickness and consists mostly of basalts. It was sampled at four sites on Kunghit Island (CK1 and 2), and in Douglas Inlet (CK3 and 4). Excellent coherence after demagnetisation occurs at three sites. At Kunghit the directions are reversed, and at Douglas Inlet they are normal. The rocks have

Fig. 1 Sampling localities in the Queen Charlotte Islands. (Geology from ref. 1.)

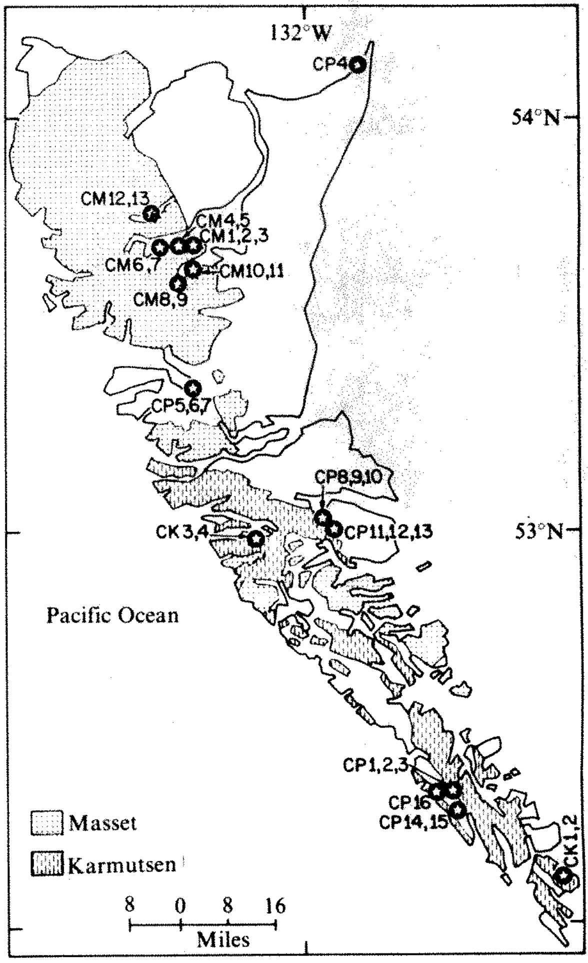

Table 1 Results of samples taken from the Queen Charlotte Islands

	*B**	*N*	Means	*k*	α°_{95}
Masset Formation directions	8	52	026, +78	81	6
Masset Formation poles	8	52	097°W, 72°N	24	11
Younger Plutons directions	5	27	338, +80	138	4
Younger Plutons poles	5	27	154°W, 70°N	90	8
Karmutsen Formation directions	3	17	359, +69	68	15
Karmutsen Formation poles	3	17	165°W, 89°N	28	24

*B is the number of sampling sites, *N* the number of cores (two specimens each), declination (long.) and inclination (lat.) of mean direction (pole) calculated irrespective of sign, *k* is the precision, and α°_{95} the circle of confidence. All specimens were cleaned in alternating fields of 30–40 mT. (Site locations and statistics may be obtained from E. Irving).

been metamorphosed to greenschist or amphibolite facies during the Late Jurassic, and the magnetisation could be of this age.

One Younger Pluton was sampled between 1 and 2 km north of Louise Narrows. The rocks are massive quartz monzonites. They give excellent results. Three sites are reversed, and two are normal. Five cores of Karmutsen basalt from the contact aureole 30 m north of the contact gave general poor overall coherence, but three other cores had reasonable end points with a mean of 345, +51, in rough agreement with directions just inside the pluton (CP11 and 13). It is difficult to estimate the horizontal plane at the time the plutons were magnetised, but there is no reason to suspect that they have been tilted and no correction has been made.

Thirteen sites were sampled from the Masset Formation on Ian Lake and Masset Inlet (Fig. 1). No coherent magnetisations were found in the basalts at sites CM1 to 3 and CM12 and 13. At the remaining sites the basalts and rhyolites yielded excellent results. Four sites have normal polarity and four are reversed. Apparent dips of the lavas vary from 0° to 10° and may be depositional so no correction has been made. The mean apparent dip is 5° to the NNW, and application of a correction would not substantially affect our conclusions.

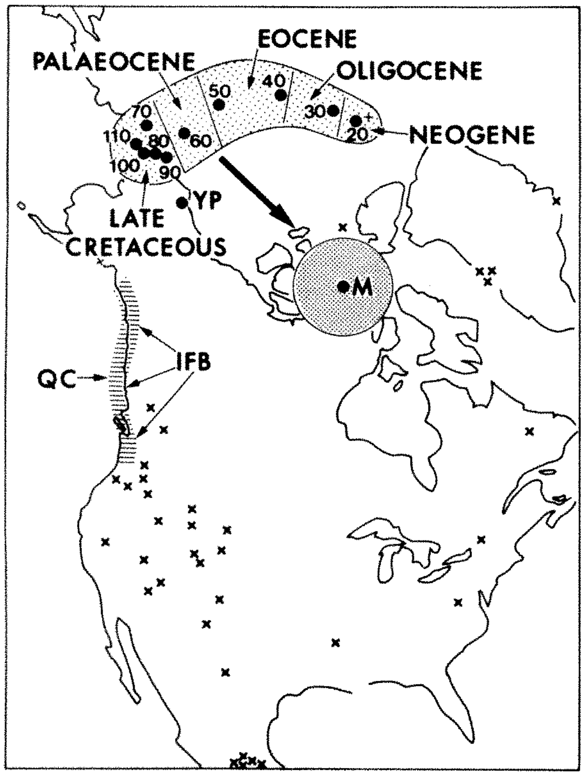

Fig. 2 The pole with its standard error for Masset Formation (M) is compared with the mean polar path for North America. The mean path is based on results obtained from the localities marked X, the results from Greenland being corrected for opening of the Labrador Sea. The mean poles, calculated as overlapping averages for the interval 110 Myr to 5 Myr, are shown, and the zone enclosing them is the envelope of their standard error circles (E. Irving unpublished data.) Standard errors are used because the significance of differences can be readily assessed; if the probable errors do not overlap then the means are significant different at probability of 95%. The present geographical pole is the cross near the Neogene pole, *QC*, Queen Charlotte Islands, *IFB*, Insular Ford Belt, *YP*, pole for the Younger Plutons.

The results are summarised in Table 1. Although the total number of results is not great (16 sites) the precisions are high. The magnetisation of the Masset Formation is considered to be Palaeocene. In Fig. 2 the pole for the Masset Formation is compared with the average pole path for the Cainozoic of a stable North America. The pole is to the SE of its expected position. An anticlockwise rotation of 53±28° brings the pole into conformity with the Palaeocene of North America, suggesting that this part of the Queen Charlotte Islands has rotated at least 25° clockwise since the Palaeocene, indicating that the Insular Fold Belt was originally approximately straight. The pole for the Younger Plutons agrees with the expected Palaeocene position. It is possible that their true age is earliest Tertiary, and that the rotation referred to above may have been completed between the extrusion of the Masset volcanics and the cooling of the Younger Plutons. Only the results from the Masset, however, provide good evidence for rotation, the data from the Younger Plutons being insufficient.

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Frequency dependence of elasticity of rock—test of seismic velocity dispersion

LINEAR theories of attenuation of acoustic or seismic waves in media, such as rocks, which are characterised by constant or nearly constant Q factors (fractional energy loss per cycle equals $2\pi/Q$), imply velocity dispersion and, therefore, frequency dependence of elasticity. The effect is small, corresponding to a change of order 1% over the period range of seismological interest (1 s to 1 h), and is consequently difficult to observe. However, it leads to an internal inconsistency in the development of earth models by inversion of free oscillation data and to discrepancies between these models and body wave data[1-3]. It is a matter of considerable geophysical interest to resolve the problem. Validity of the linearity assumption has been questioned[4] but we are now observing elliptical hysteresis loops in basalt and granite samples subjected to sinusoidal strains of order 10^{-6} and this is strong evidence that attenuation does become a linear phenomenon at low strain amplitudes. But whether or not perfect linearity applies, a direct demonstration of body wave dispersion is the most satisfying indication that earth model studies need revision.

The requirement of causality in linear attenuation theories (which we propose to review in a separate paper) leads to the relationship between phase velocity $v(\omega_0)$ at angular frequency

ω_0 and an integral involving the attenuation coefficient, $\beta(\omega)$

$$\frac{1}{v(\omega_0)} = \frac{1}{c} - \frac{2}{\pi} P \int_0^\infty \frac{\beta(\omega)}{\omega_0^2 - \omega^2} \, d\omega \tag{1}$$

where P indicates the Cauchy principal value of the integral, c is a constant (phase velocity at $\omega \rightarrow \infty$) and a plane wave of any frequency ω decays with distance x as $\exp(-\beta x)$. For the present purpose, it suffices to assume that the behaviour of β at very high frequencies is such that the integral in equation (1) converges but that over a wide range of frequencies about ω_0, β is proportional to ω, which is very nearly equivalent to the constant Q assumption because

$$\beta = \omega / 2Qv \tag{2}$$

the velocity dispersion being slight. Then it follows that, to first order in Q^{-1}, values of elastic modulus μ (real part of the complex modulus), at frequencies ω_1 and ω_2, are related by

$$[\mu(\omega_2) - \mu(\omega_1)]/\mu(\omega_1) = (2/\pi Q) \ln(\omega_2/\omega_1) \tag{3}$$

The derivation of equation (3) demonstrates the equivalence for practical purposes of the alternative empirical linear theories[5-7], which differ only in the assumptions regarding the high frequency behaviour of β, as well as the mechanistic theory based on logarithmic creep[8-11]. The logarithmic dependence of elasticity or velocity on frequency is characteristic of frequency independent Q; other assumptions regarding $\beta(\omega)$ lead to quite different results, so that the direct observations which we report here, confirming the validity of this relationship, also support the constant Q assumption. The logarithmic dependence seems to have been quoted first by Kolsky[12], but we have been unable to find the earlier derivation. This dispersion law also satisfies the observations of pulse shape in acoustic experiments[12,13] and in particular the increase of pulse rise time τ with time of travel and Q for the path[13]

$$\Delta\tau \approx 0.5 \int Q^{-1} \, dt \tag{4}$$

Our experiment is similar in principle to that of McKavanagh and Stacey[14], who measured the mechanical hysteresis of rock cores under cycled axial compressions. Our specimens are tubes of rock of length about 280 mm, diameter 44 mm and wall thickness 5 mm, and are subjected to torsion. The use of thin walled tubes ensures that the strain, which is pure shear, is approximately uniform through the material. The specimen under examination is connected to a standard tube, either of high Q steel or of fused quartz, subjected to the same torque and used to infer the stress. Rings, fixed about 20 mm from both ends of each specimen and standard, are used for mounting capacitance plates, which are centred about 200 mm from the specimens. Relative displacements of the plates record the twist of the specimen between its mounting rings. Balanced sets of plates are mounted on opposite sides of each specimen to minimise effects of asymmetry and thermal drift. Measurements of capacitance are made by means of ratio transformer bridges[14,15] with rapid response times (10^{-3} s) which avoid the appearance of any instrumental phase lag in the measured strains.

Small stresses are applied by means of loudspeaker coils mounted on a lever arm connected to one end of the standard specimen and driven by current from a low frequency sinusoidal oscillator. Strains in the rock specimen and standard are recorded on an X–Y recorder, giving, in effect, a stress–strain plot as the strain is cycled. With the granite and basalt specimens which we have used in this experiment, the hysteresis loop is barely apparent. However, by subtracting the two bridge outputs from one another (specimen minus standard) in a difference amplifier, with independently controlled gains on the two inputs, and further amplifying the difference signal, we can plot directly the departure of specimen strain from linearity with strain of the standard. This appears as a loop of adjustable width, whose area is a measure of the hysteretic loss in the specimen, relative to the standard. The slope of the axis of this loop is a very sensitive indicator of the elastic modulus. The fact that we are able to observe such fine details of loop shape at strain amplitudes as low as 10^{-6} (hopefully lower with improvements now planned) is due to the high sensitivity and wide linear range of the capacitance displacement transducer[14-17].

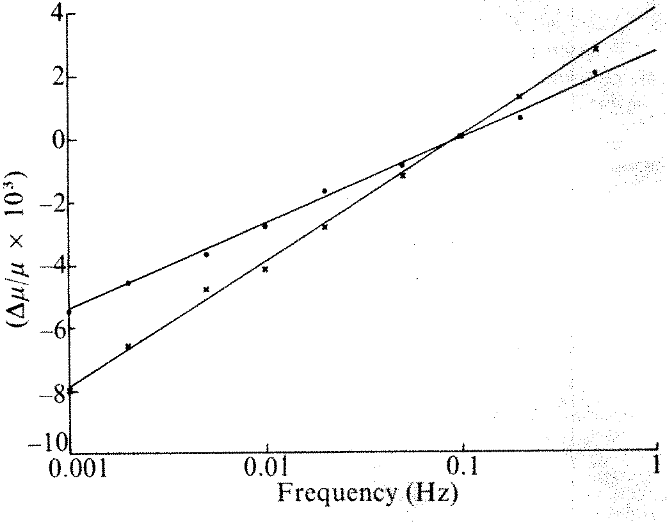

Fig. 1 Frequency dependences of rigidity moduli of basalt and granite specimens over the range 0.001–0.5 Hz relative to values of 0.1 Hz, which was used as a reference frequency in repeated comparisons. ●, Basalt, $Q = 590$, strain amplitude $= 2.6 \times 10^{-6}$. ×, Granite, $Q = 320$, strain amplitude $= 2.9 \times 10^{-6}$. By equation (3) the gradients correspond to Q values of 546 and 371 respectively.

We have plotted hysteresis loops for specimens of basalt and granite and found that the elastic moduli vary with frequency precisely in the manner of equation (3) over the period range 2–1,000 s, which covers much of the seismic frequency band. Figure 1 gives plots of the data, represented as the fractional changes in rigidity modulus relative to the values at 0.1 Hz. The gradients of the two graphs do not agree perfectly with the theoretical values obtained from the measured Q values by equation (3). The discrepancies may arise because the standard specimens were not completely lossless or from frequency dependence of Q, although the measured Q values were independent of frequency within the accuracy of our measurements.

The hysteresis loops of both specimens were of elliptical form, not cusped at the ends, indicating that within the precision of these observations the attenuation mechanism is linear. This was not the case with the sandstone specimen examined by McKavanagh and Stacey[14], even down to strains of 10^{-6}, or with granite and basalt specimens at strains of order 3×10^{-5}. It seems that the strain amplitudes used in the present experiment are at the upper limit of the linear range. Further instrumental improvements are required to examine with the same precision Q and modulus changes at strain amplitudes below 10^{-6}.

If we accept the validity of equation (3) then it should be possible to adjust earth free oscillation data by using measured Q's of the identified modes to remove the effect of frequency dependence of elasticity. Comparison with body wave data also requires recognition of the fact that in the presence of dispersion, as represented by equation (3), the group velocity v_g of a body wave is related to phase velocity by

$$v_g = v(1 + 1/\pi Q) \tag{5}$$

to first order in Q^{-1}. This is the observed velocity in the case of a body wave, but free oscillations effectively measure phase velocity, which for the mantle is of order 0.1% smaller.

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Carboniferous unconformity in southern Africa

PALYNOLOGICAL investigations indicate that the Dwyka Tillite of southern Africa is probably entirely of Early Permian age and that the Carboniferous System is absent from this region. Early investigations into continental drift[1] indicated that there were identical late Palaeozoic successions in the southern continents. In this letter, this identity is shown to be incorrect in some respects and, as yet, unsubstantiated in others.

The Dwyka Tillite is most fully developed in the Karoo Basin of South Africa (Fig. 1) although occurrences are known as far north as the equator. The unit reaches thicknesses of up to 1,000 m in a deep sedimentary trough which extends along the southern margin of the basin. There are few reliable palaeontological data for the age of the unit. A small marine invertebrate fauna from South-west Africa was dated as Early Permian[2], and Early Permian miospore assemblages have been reported from Zaïre[3,4], Rhodesia[5], and South-west Africa[6]. A miospore assemblage from above the tillite in the northwestern part of the Karoo Basin was tentatively assigned to the Late Carboniferous[7], but the forms present could equally well support an Early Permian age. There are no reasons to believe that any part of the unit is younger than Early Permian.

Various ages have been reported for the Dwyka Tillite: Early Carboniferous, possibly Late Devonian, to Early Permian[8]; Late Carboniferous[1]; Early Permian[9]. The principal argument for a Carboniferous component for the age rests on an apparent conformable contact in the southern trough between the Dwyka Tillite and the underlying Waaiport Formation of Late Devonian or Early Carboniferous age[8,10]. The Early Permian determinations noted above were all from outside the region of maximum deposition and may represent only later stages.

Within the southern trough, palynomorph microfossils have been badly damaged by heat and tectonism. Three localities (Fig. 1) yielded recognisable specimens. (1) At Elandsvlei, the Dwyka Tillite consists of four sedimentary cycles which can be traced south into the trough and thence eastwards for about 100 km. A sample was taken from the base of the lowest cycle. (2) At Sambokkraal, a deep borehole penetrated 650 m of Dwyka Tillite. A sample of core was taken 1 m above the base of the unit. (3) At Waaipoort, the Dwyka Tillite uncomformably overlies the Kommadagga Subgroup (Fig. 2). A sample was taken from the Miller Diamictite, the lowest unit in the subgroup.

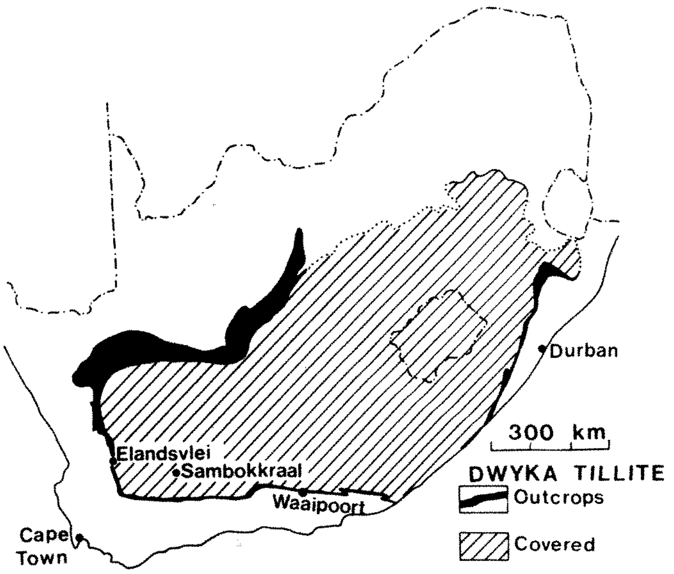
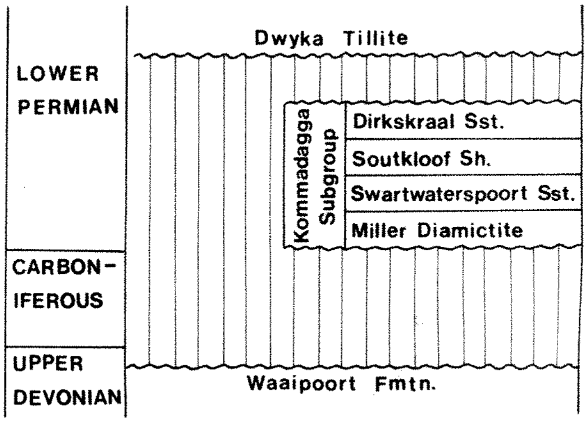


Fig. 1 Distribution of Dwyka Tillite in the Karoo Basin.

The samples should be uncontaminated. The samples from Elandsvlei and Waaipoort were collected from outcrops and the Sambokkraal borehole was cored throughout. The lithologies are too distinctive to be confused with those of any other units in the region. There is insufficient jointing to permit stratigraphic leakage. Normal precautions against contamination were taken in the laboratory.

All of these samples contained bisaccate pollen grains (Fig. 3*a–e*). Although the grains were badly damaged, it was possible to determine that some, at least, had striated central bodies (Fig. 3*a*, *b*, *d*). Many large monosaccate grains were also present (Fig. 3*f*). Early Permian assemblages are characterised by monosaccate and striated bisaccate pollen. The monosaccate grains were too badly preserved to permit identification even to the generic level and similar forms are known from the Late Carboniferous. Striated bisaccate pollen, however, did not appear until well into the Stephanian. Since the sample from the Miller Diamictite was taken 400 m below the Dwyka Tillite, separated from the tillite by an angular unconformity, the base of the tillite is probably above the Permo–Carboniferous boundary. Nevertheless, the possibility exists

Fig. 2 Relationship of Dwyka Tillite to underlying units, southern Karoo Basin.



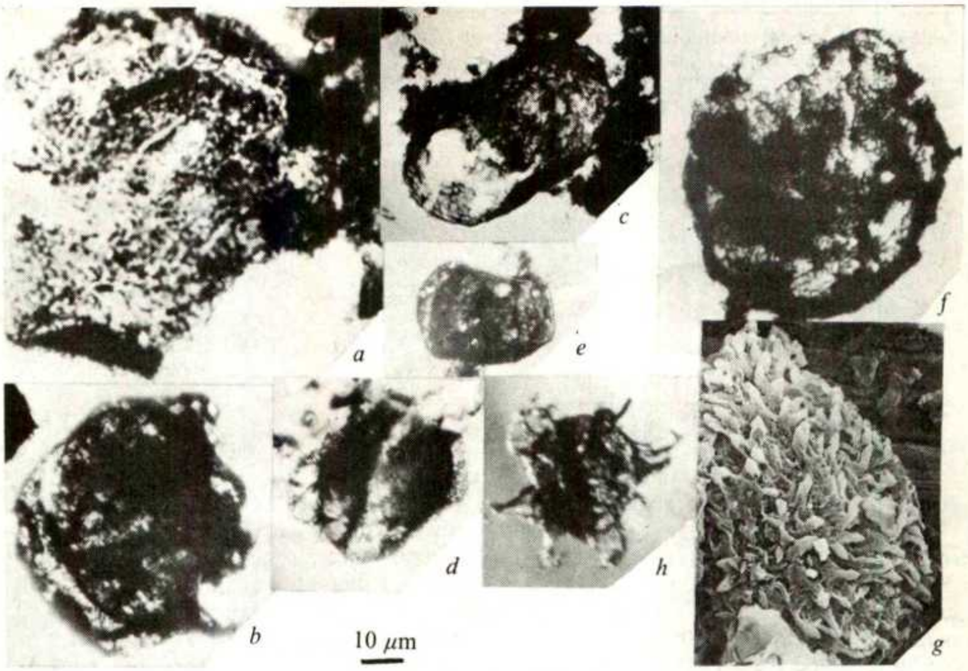


Fig. 3 *a*, Bisaccate pollen, Dwyka Tillite, Elandsvlei. *b*, *c*, Bisaccate pollen, Miller Diamictite, Waaipoort. *d*, *e*, Bisaccate pollen, Dwyka Tillite, Sambokkraal. *f*, Monosaccate grain, Miller Diamictite, Waaipoort. *g*, *Dibolisporites echinaceus*, Waaipoort Formation. *h*, ?Acritarch, Dwyka Tillite, Sambokkraal. Note striations on *a*, *b*, and *d*.

that Dwyka deposition was initiated in the latest Carboniferous.

At Waaipoort, the Kommadagga Subgroup is underlain by the Waaipoort Formation which, further to the west, directly underlies the Dwyka Tillite itself (Fig. 2). Plant megafossils from the Waaipoort Formation have given ambiguous Devonian or Carboniferous ages. Palaeoniscoid fish, from nodules collected about 200 m below the top of the formation, were dated as Visean on the basis of a broad resemblance at the familial level with Carboniferous fish of Britain[11]. A residue extracted from one of these same nodules contained carbonised Devonian spores including such species as: *Acinosporites acanthomamillatus* Richardson, *Ancyrospora langii* (Taugourdeau–Lanz), *Contagisporites optivus* var. *vorobjevensis* (Chibrikova), and *Dibolisporites echinaceus* (Eisenack) (Fig. 3*g*). Many of the same Devonian species were found reworked into the Miller Diamictite and some Devonian forms were seen in the Dwyka Tillite at Sambokkraal. The assemblage from the Waaipoort Formation indicates a Givetian or possibly Frasnian age. It is unlikely that the 200 m of the Waaiport Formation above the sample horizon would extend completely through the Upper Devonian into the Carboniferous. No other Carboniferous rocks have been reported from subequatorial Africa.

There are insufficient data to permit reliable age determinations for the late Palaeozoic diamictites of Antarctica or the Parana Basin of South America. Those of India and Australia are of Early Permian age, with possibly some Late Carboniferous present in both regions. Thus, the Late Palaeozoic diamictites of India, Australia and southern Africa were all deposited within the same time interval, from latest Carboniferous to Early Permian. This interval is too long, and the ages too imprecise, to determine whether or not the units were actually synchronous.

No Carboniferous is known from Antarctica, though this may be due to incomplete exploration. There is Lower Carboniferous in India and fairly complete Carboniferous successions occur in Australia and South America. Thus, the late Palaeozoic history of Gondwanaland is more complex than has hitherto been indicated.

Some possible acritarchs (Fig. 3*h*) were seen in the Dwyka Tillite at Sambokkraal. No such forms were seen in the Waaipoort Formation and hence they are unlikely to be reworked. It has been postulated that, in the southern trough, the Dwyka Tillite was deposited from a floating ice shelf[10], which might account for the presence of such forms, but such a model is difficult to reconcile with the presence of gymnosperm pollen in the same sample. These samples were diamictite, not interbedded shale. Pollen has been recovered from Dwyka diamictites in other parts of the Karoo Basin and in Rhodesia[5]. In the south-western part of the Karoo Basin, the diamictite has an average organic carbon content of about 0.1%, mostly as terrestrial plant fragments. These data seem incongruent with accepted models of sedimentation for the Dwyka Tillite.

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Silicate and the aqueous corrosion of a high-magnesium alloy

Following Butler and Mercer's demonstration[1] that silicate derived from dissolution of glass suppresses the corrosion of mild steel in distilled water at 90 °C, we have compared the results of exposure of Magnox AL 80 (a magnesium alloy containing 0.8% of aluminium) to solutions with and without silicate contamination. This showed that silicate enhanced corrosion in alkaline solution, the effect being greater the higher the temperature. Both magnesium hydroxide and an hydrated magnesium aluminium silicate seem to be formed in the corrosion reaction. During our extensive studies[2] of the aqueous corrosion of Magnox AL 80, care has been taken to store and use alkaline solutions in plastic containers since dissolution of borosilicate apparatus readily causes contamination[3]. A few tests have, however, been conducted with

Table 1 Experimental conditions for corrosion tests on Magnox AL 80 in sodium hydroxide solutions (*p*H 11–12)

Test number	Dissolved silicon concentration (mg kg^{-1})	Temperature (°C)	Test duration (d)
1	100	60	120
2	0	105	0.17
3	130	105	0.25
4	0	121	0.1
5	130	121	0.1 1.0

solutions known to contain silicate and data from these form the basis of our observations.

Corrosion experiments were carried out using specimens with surface areas of a few cm^2 cut from Magnox sheet, degreased, and chemically etched in 2% weight/volume citric acid solution for 5 min. The specimens were immersed in sodium hydroxide solutions (prepared from double distilled water) with *p*H values between 11 and 12. In these solutions magnesium spontaneously forms a passivating film[4]. Conditions of the tests in which the behaviour with and without silicate in solution could be contrasted directly are summarised in Table 1.

In Test 1, specimens were exposed to solution contained in a soft-glass tank, the *p*H of the liquid being maintained above 11 by occasional addition of fresh hydroxide solution. The dissolved silicon content was variable but averaged about 100 mg kg^{-1}. The solution for Test 5 initially contained 130 mg kg^{-1} of silicon produced by etching of the flask in which it was prepared. The same silicon concentration in the form of sodium metasilicate was added to uncontaminated hydroxide for Test 3.

Previous measurements over a range of temperatures enabled the corrosion rate in silicate-free solution at 60 °C to be estimated accurately. Measurements at the higher temperatures were made with Magnox samples in PTFE liners within small stainless steel autoclaves each of which was fitted with a pressure transducer and chromel–alumel thermocouple. Average corrosion rates were calculated from the specimens' weight gains, allowing for corrosion product spalling, and assuming the product to be magnesium hydroxide[2]. Independent checks of the overall metal losses derived from the pressure of gas generated in Tests 2, 3 and 5 gave values close to those estimated gravimetrically.

With silicate-free solution our results were consistent with those of Masterson and Harrison[5] for etched Magnox at 40 °C and with data of Blanchet *et al.*[6] for the magnesium alloy GA3Z1 between 65 and 95 °C. In comparison, our tests suggest that corrosion rates for Magnox are a factor of 3 lower than those quoted by Staesche[7] for a Mg–Mn alloy, although the temperature dependence is very similar. Greenberg and Ruther's experiments[8] on alloy AZ 31 gave rates approximately twice what we might expect for AL 80.

At 60 °C average corrosion rates of Magnox in solutions containing silicate were indistinguishable from those in uncontaminated solutions, while at 105 °C the average rate was twice as great, and at 121 °C four times higher, than in silicate-free solutions after similar periods of exposure. This enhancement factor for silicate solution had doubled at the end of Test 5. These results contrast with a fivefold decrease in the corrosion rate of mild steel in distilled water at 90 °C produced by 4.7 mg kg^{-1} of dissolved silicon as reported by Butler and Mercer[1].

On completion of Test 5 the concentration of dissolved silicon had dropped to 0.1 mg kg^{-1}. The possibility that silicate may have reacted with the Magnox was suggested by results from Test 1. Specimen weight gains during this test were incompatible with the weight losses measured after chemically stripping the corrosion product at the end of the experiment unless a magnesium silicate formed part of the corrosion product.

Energy dispersive X-ray analysis (EDAX) showed silicon on specimens from Test 1, and subsequently both EDAX and α-particle back-scattering analysis[2] proved that some samples from high temperature experiments contained silicon in their corrosion products.

An infrared reflectance spectrum was readily obtained with the specimen from Test 3 (sample *b*) which had a thin (1.8 μm) transparent film on a smooth metal surface. This spectrum was compared with one recorded for a corrosion product formed in silicate-free solution—sample *a* (specimen 4 of ref. 2). Figure 1 shows that absorption bands due to bound water (~3,250 cm^{-1}) and to silicate (at ~1,010 and 650 cm^{-1}) are present in addition to those of magnesium hydroxide. From the wavelengths of these bands it is inferred that a polymeric hydrated silicate had been incorporated into the corrosion film.

The corrosion film on the specimen from Test 5, being 30 times thicker, probably contained much more silicate than the product on sample *b*, but a satisfactory infrared spectrum could not be obtained from it as very little incident radiation was reflected from the rough surface. An X-ray powder diffraction spectrum of the corrosion product from this specimen showed the presence of both magnesium hydroxide and a magnesium aluminium silicate of the chlorite type, rather than a simple magnesium silicate.

These results demonstrate that silicate contamination can enhance the corrosion of Magnox AL 80 in sodium hydroxide solutions above 60 °C. Even at this temperature gravimetric data suggest participation of silicate in the process, and scanning electron microscopy–EDAX indicates

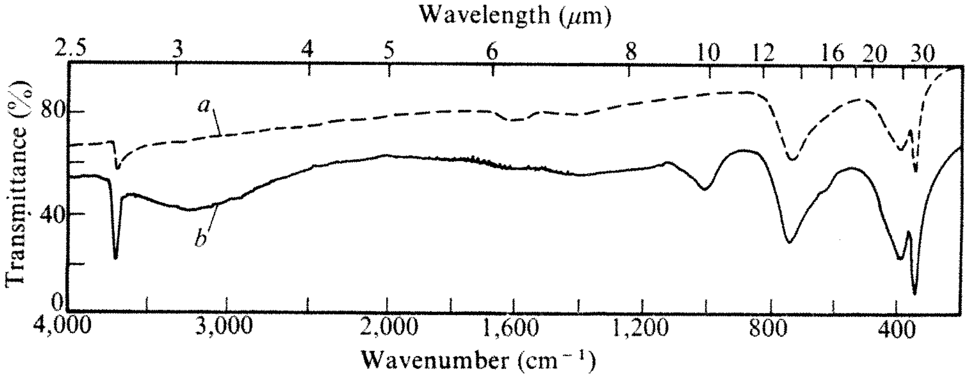

Fig. 1 Infrared reflectance spectra of Magnox specimens coated with thin films of *a*, magnesium hydroxide; *b*, magnesium hydroxide incorporating silicon (Perkin-Elmer 577 grating spectrometer; attenuated air reference).

the preferential formation of a magnesium silicate at grain boundaries. The X-ray diffraction evidence implies that the aluminium content of the alloy, although small, influences the chemical products formed in the corrosion reaction.

It has not been possible here to report on the effect of solution composition on the variation of corrosion rates with time which we have observed, both in experiments involving continuous measurement of gas evolution and by electrochemical techniques. Detailed study of the complex kinetics of the reaction of Magnox with water, its enhancement by silicate and inhibition by fluoride is continuing in our laboratory, and will be reported in due course.

We thank Dr G. C. Allen for communicating his detection of a magnesium silicate by infrared spectroscopy, Dr E. Metcalfe for X-ray analysis of corrosion products and Dr D. A. Hilton for helpful comments. This work is published by permission of the Central Electricity Generating Board.

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Thermoluminescence from γ-ray irradiated normal paraffins

IN recent years, an increasing number of papers have described the trapping mechanisms in organic materials including polymers evaluated by thermoluminescence measurement. Several authors have attempted to correlate the phenomena of thermoluminescence glow peaks and the molecular motions in polymers[1,2]. Such studies give useful information on transitional motions which may be responsible for thermal glow from the irradiated organic compounds[3]. This letter presents some of the results obtained from a study of thermoluminescence in normal paraffins above room temperature.

The samples used were commercial normal paraffins in the range *n*-eicosane (C_{20}) to *n*-nonacosane (C_{29}) which were purified by recrystallisation from acetone and/or ethanol hot solution several times. The actual purities estimated from the melting curves were 99.6% for C_{24} and above 99% for the others. All samples were warmed from room temperature to above their melting temperature after irradiation by 0.5 Mrad γ-ray doses, obtained from ^{60}Co at dry ice temperature in air. The luminescence detection apparatus consisted of a photomultiplier (HTV R292) contained in a light-tight box. The temperature was measured by copper–constantan thermocouples attached to the surface of the sample. The calorimetiric measurements were made using a Parkin Elmer DSC II type calorimeter. A scanning speed of 5 °C min^{-1} was employed.

Figure 1 illustrates a typical thermoluminescence glow curve obtained from irradiated C_{24} and C_{28}. Samples were kept in the dark at room temperature after irradiation and during warming. When a sample had given a glow curve, no further light was emitted if it was immediately cooled and reheated. Sharp peaks having very narrow half-line widths of around 0.5 °C were observed in the curves of all the samples, some of which have another broad emission peak on the lower temperature side as shown in Fig. 1. These broad peaks decay relatively fast, while the sharp ones were stable and remained constant in spite of varying with warming rate ranging from 1 to 20 °C min^{-1}.

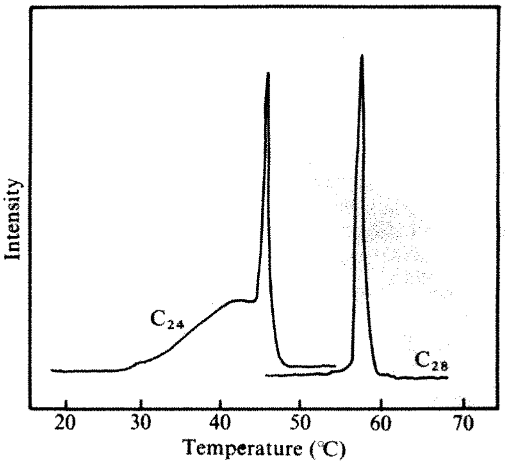
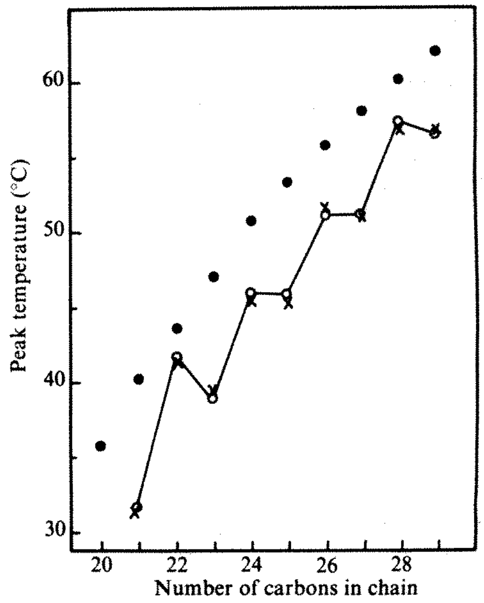


Fig. 1 Thermoluminescence glow curves of irradiated C_{24} and C_{28}. A heating rate of 5 °C min^{-1} was used.

It has been clearly demonstrated that a large number of longer paraffins exhibit first order solid state transitions (see Broadhurst[4]). In general, calorimetric measurements are effective in analysing thermodynamic phase transitions. The glow peaks and DSC peaks obtained are plotted against carbon numbers in Fig. 2. It should be noted here that the glow peaks for all samples were in good agreement with solid state transition points. No significant emission was observed at melting. From these results, thermoluminescence at the transition point is not given by simple Boltzman-type variation with temperature.

Equimolar melts of two paraffins with different carbon numbers were also examined. The DSC curve of such a

Fig. 2 Plot of glow and DSC peak temperature as a function of number of carbons in chain. ×, glow peak; ●, melting point in DSC; ○, transition point in DSC.



mixture has two peaks; one was assigned to the melting and the other, of greater temperature band width was assigned to the solid-state transition in the order of descending temperature. Comparing DSC spectra with thermoluminescence peaks, it is also apparent that for binary mixtures, light emission occurs at the solid-state transition point.

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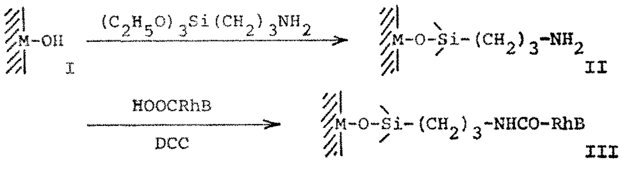
Photocell using covalently-bound dyes on semiconductor surfaces

ONLY TiO_2,[1] SnO_2,[2] and $SrTiO_3$,[3,4] which do not undergo decomposition upon irradiation when used as photoelectrodes in aqueous media, are stable enough to be used as practical solar energy conversion and hydrogen production devices. The band gaps of these *n*-type semiconductors correspond to the energy of photons in the ultraviolet region. Solar energy reaching the Earth's surface has, however, a spectrum distribution in the longer wavelength range and cannot, therefore, be used effectively by these semiconductors[5,6]. Spectral sensitisation would solve this problem by extending the sensitivity of the electrochemical photocells to longer wavelengths. In applying this technique to a real photocell another problem may arise in that most of the solar energy is absorbed by the dye molecules in the solution rather than by the dye molecules adsorbed at the electrode–solution interface, though only the excited adsorbed dye molecules can contribute to sensitisation[7–9]. To cope with this we have developed a new type of photocell[10]. In this, the chemically modified SnO_2 or TiO_2 electrode, with rhodamine B as a sensitiser, was in contact with the transparent electrolyte solution containing a reducing agent as a supersensitiser. In this communication, further developments of this subject are described, especially, the correlation between the efficiency of sensitisation and the structure of the modified layer, which gives us the criteria for more successful chemical modifications of electrode surfaces.

In the previous work, the following surface modification procedure was used to synthesise an amide-linked rhodamine B electrode.



$$\text{M-OH (I)} \xrightarrow{(C_2H_5O)_3Si(CH_2)_3NH_2} \text{M-O-Si-}(CH_2)_3\text{-}NH_2 \text{ (II)} \quad (1)$$

$$\xrightarrow[\text{DCC}]{\text{HOOCRhB}} \text{M-O-Si-}(CH_2)_3\text{-NHCO-RhB (III)} \quad (2)$$

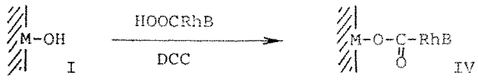
M = Sn or Ti, DCC = dicyclohexylcarbodiimide,

HOOCRhB = rhodamine B

The sensitised photocurrent observed at the amide-linked electrode was less than that observed at the conventional untreated electrode in contact with the dye solution.

Since then, we have tried to increase the surface coverage of the amide-linked rhodamine B (III) and ultimately to increase the sensitised photocurrent by improving yields of the silanisation reaction (I→II). It was found, however, that the observed sensitised photocurrent on the modified electrode (III) decreased, against our expectation, with an increase in the surface coverage of aminopropyl derivative (II)[12]. This led us to assume that rhodamine B covalently bound via amide bond (III) cannot contribute to the spectral sensitisation, and the observed photocurrent on the amide-linked electrode prepared previously can be attributed to rhodamine B directly bound on the surface via ester bond (IV) with the surface hydroxyl group (I) which remains unreacted with γ-aminopropyltriethoxysilane.

Consequently, the following dehydrative coupling of a carboxyl group of rhodamine B with a surface hydroxyl group of the untreated electrode was tried using dicyclohexylcarbodiimide (DCC) as a dehydrating agent[11].



$$\text{M-OH (I)} \xrightarrow[\text{DCC}]{\text{HOOCRhB}} \text{M-O-}\overset{O}{\overset{\|}{C}}\text{-RhB (IV)} \quad (3)$$

As expected, a sensitised photocurrent spectrum was obtained with the ester-linked rhodamine B electrode and was exactly the same as that observed previously on the amide-linked electrode as shown in Fig. 1. Furthermore, the sensitised photocurrent is about two orders of magnitude

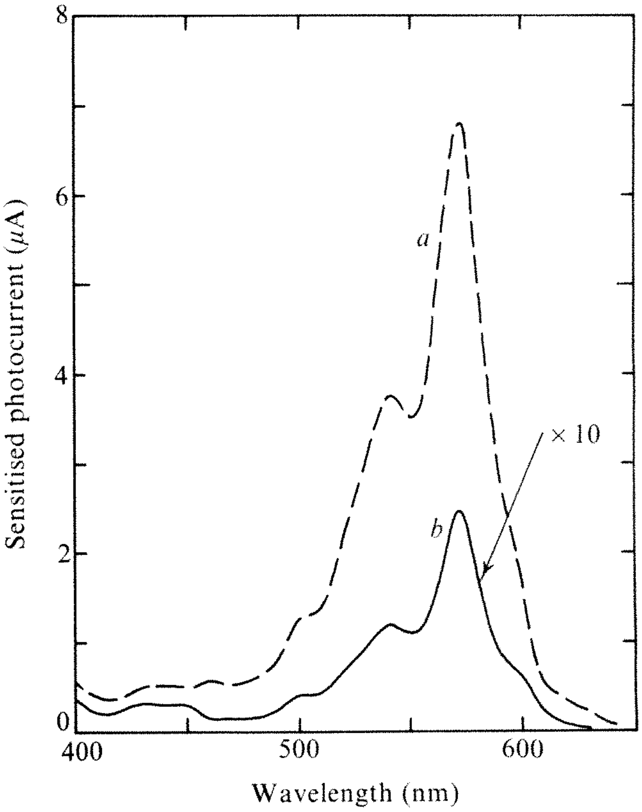


Fig. 1 Spectral dependence of the photocurrent at the chemically modified SnO_2 electrodes with rhodamine B. The cell design used for the photoelectrochemical studies was the same as in the previous paper[10]. The optically transparent electrode was polarised anodically at +0.4 V against a saturated calomel electrode (SCE) and illuminated through the electrode (as shown in Fig. 1 of ref. 10). *a*, Photocurrent spectrum sensitised by a sub-monolayer of ester-linked rhodamine B in contact with a Britton–Robinson buffer (*p*H = 3.9) containing hydroquinone (10^{-2} M) as a supersensitiser. *b*, Photocurrent spectrum observed previously at the amide-linked rhodamine B electrode in contact with aqueous 0.2 M Na_2SO_4 containing hydroquinone (5×10^{-3} M), which is magnified 10 times for comparison.

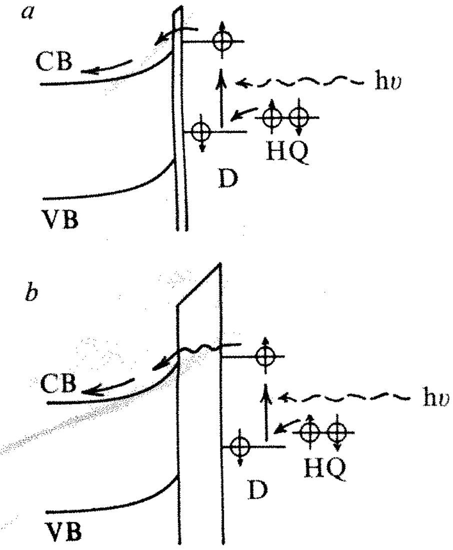


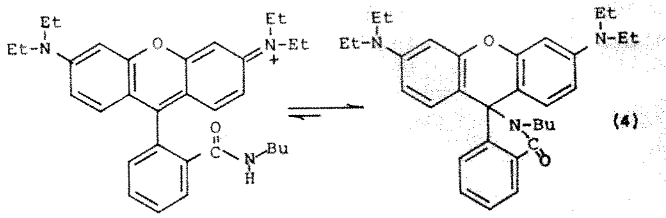
Fig. 2 Schematic presentation of supersensitisation at semiconductor-covalently-bound dye-reductant solution interfaces under anodic bias: D, dye molecule covalently bound on a semiconductor as a sensitiser; HQ, hydroquinone in solution as a supersensitiser; CB, conduction band; VB, valence band. *a*, Ester-linked dye electrode where π electronic system of dye is close to the semiconductor surface. *b*, Amide-linked dye electrode via amino-alkylsilane where π electronic system of dye is apart from the semiconductor surface and a non-conducting alkyl chain intervenes between semiconductor and dye.

greater on the ester-linked electrode than the photocurrent observed previously on the amide-linked electrode.

The difference in distance of the conjugated π electronic system of rhodamine B from the electrode surface probably accounts for the marked difference in efficiency of the electron injection from excited dye molecules to the conduction band of semiconductor for these ester- and amide-linked electrodes, as shown in Fig. 2. (As these surfaces are chemically predictive, we can evaluate more correctly the height and width of the energy barriers from the properties of binding species such as electron affinity and length of alkyl chains compared with those of the vapour-deposited insulator film such as metal oxides.) In this picture, intervention of the non-conducting alkyl chain in the amide-linked electrode is described as an energy barrier which inhibits the electron injection. This explanation can be supported by the observed difference in electrochemical reactivity of chemisorbed alkenyl hydroquinone on platinum having various lengths of intervening alkyl chain[13] and by the following observation.

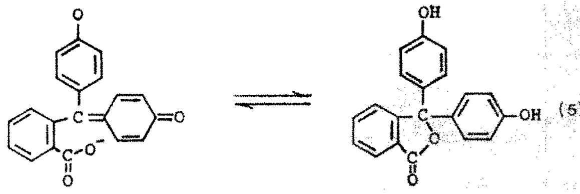
If a monolayer of non-conducting compound at the electrode–solution interface acts as the energy barrier, the electron injection from the dye molecules adsorbed physically at the electrode–dye solution interface should be also inhibited by the presence of such layers. In Fig. 3, a sensitised photocurrent spectrum observed at the aminopropyl modified electrode (II) in contact with the rhodamine B solution is compared with that obtained with the unmodified electrode (I). A pronounced lowering in the sensitised photocurrent was observed by the introduction of nonconducting layers on electrode surfaces.

Parallel to the above experiments, which elucidate why the amide-linked rhodamine B cannot contribute to the spectral sensitisation, the conventional synthesis in solution of the amide and ester derivatives of rhodamine B with butylamine and ethanol was carried out to check the variation of their absorption spectra by derivation. These derivatives were obtained in satisfactory yields by using DCC under the same experimental conditions as used for the surface synthesis. The absorption spectrum of aqueous solution of the ethyl ester is very similar to that of rhodamine B, whereas the butyl amide is surprisingly almost colourless in most solvents. This is because the amide derivative is stable in the lactam form rather than the amide form as shown in equation (4).



(4)

Thus, the deep red colour arising from the quinoid form in the xanthane structure of rhodamine B disappears due to lactam formation of the amide derivative, seen similarly in the acid–base equilibrium of phenolphthalein.



(5)

Because the amide-linked rhodamine B loses colour, an insulator model is unnecessary in this special case to explain the lowering of sensitisation efficiency of amide-linked rhodamine B. It is very important, however, to clarify how the intervening non-conducting layer affects the spectral sensitisation process in view of an important role of lipid membranes in photo-oxidation of water[14,15]. Probably the chemical modification approach as illustrated in this paper is the best method at present to tackle the problem.

In conclusion, if the chemical modification of electrode

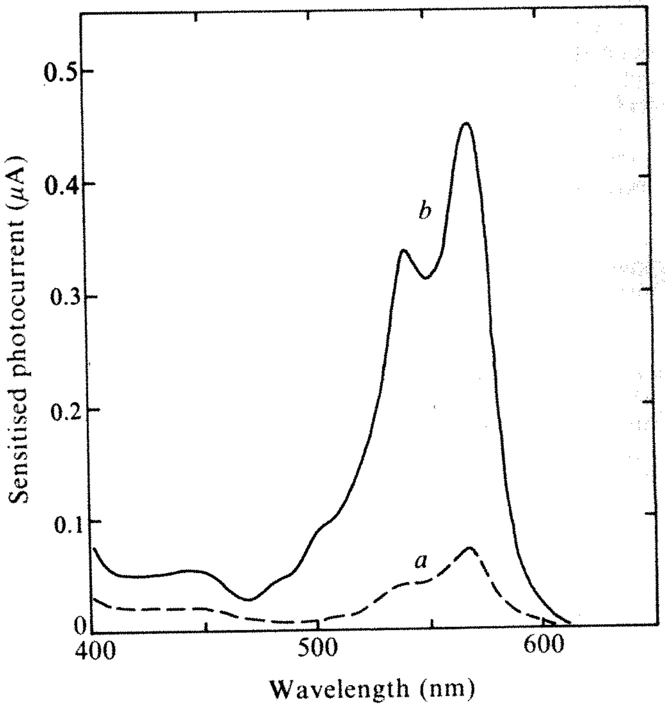


Fig. 3 Spectral dependence of the photocurrent at the electrode–dye solution interface. The conditions for photoelectrochemical measurements were similar to those described in Fig. 1. *a*, Photocurrent spectrum observed at the aminopropyl modified SnO_2 electrode in contact with a rhodamine B solution (5×10^{-5} M rhodamine B+5×10^{-3} M hydroquinone in 0.2 M Na_2SO_4). *b*, Photocurrent spectrum at the conventional unmodified SnO_2 electrode in contact with the same solution as described in *a*.

surfaces are applied to the solar cells, the sensitisers should be bound as close to the electrode surfaces as possible in order to increase the efficiency of spectral sensitisation. Care must be taken as to how the optical properties of sensitisers are changed by the modification reactions.

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Spanish rock art depicting honey gathering during the Mesolithic

THE end of the Upper Palaeolithic and the subsequent Mesolithic of western Europe coincided with a general change of climate, flora and fauna. Throughout this time the nomadic hunters and food gatherers continued their former activities and perfected their techniques in a changing environment, before the introduction of agriculture and the change to a sedentary way of life. In Spain, this period is associated with a type of rock art known as 'art of the Spanish Levant', because it occurs only within a limited area of the mountainous coast of eastern Spain; it is provisionally dated approximately from 8,000 to 2,000 BC. Its outstanding features are the appearance of motion and the fact that the central figures are mainly human, around which animal figures are grouped. We report here a scene we discovered last year at one of the most important Levantine rock-art zones, the Barranco de la Valltorta (Castellon), which is a dried out, deep and eroded river bed. It shows wild honey gathering activities and depicts the first definite ladder constructed of side ropes, with visibly rigid intersecting rungs, which are straight and even, and which must be sticks prepared for this purpose.

A stretch of about 7 km of the Valltorta contains 14 rock-art sites, totalling 39 painted rock shelters, located on both banks of the ravine (Fig. 1); these were all discovered in 1917. The paintings of the left bank have been recorded in part and published[1,2,5], while those of the right bank, located on steep cliffs and very difficult to reach, were reputed to contain only meaningless remains of paintings. During a study of the origins and development of Levantine rock art, we have checked and redrawn all known sites. On the right bank we found some paintings which could still be interpreted and correlated because the only damage they had sustained was due to frost and natural erosion. Difficulty of access had protected them from the moistening and rubbing which has defaced so many Levantine rock shelters.

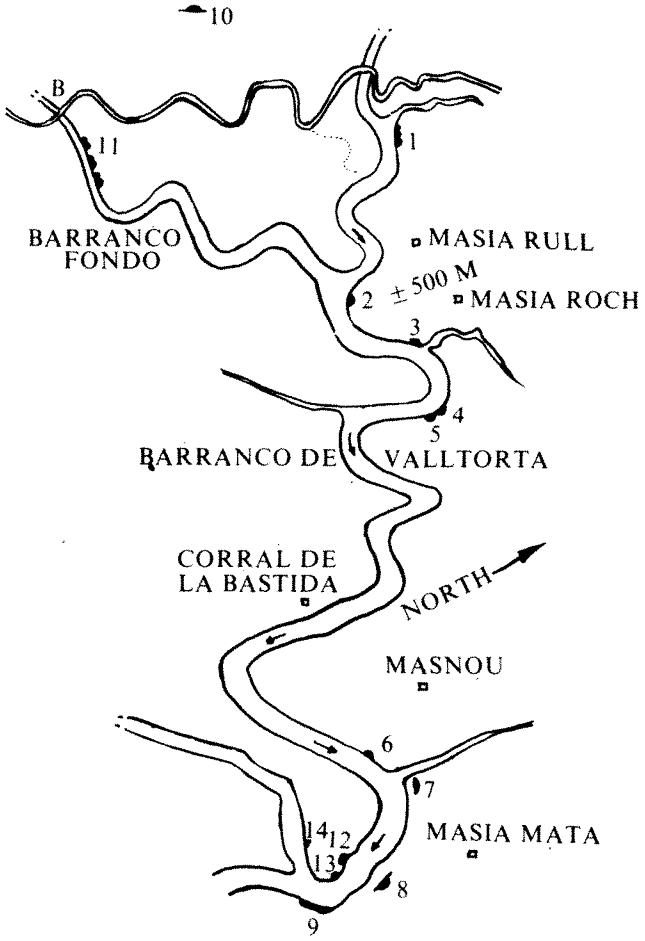

Fig. 1 The Barranco de la Valltorta and its 14 Levantine rock art sites: 1, El Civil; 2, Cueva dels Tolls Alts; 3, Cueva Rull; 4, Els Caballs; 5, Cueva del Arco; 6, Mas d'en Josep; 7, Cueva Alta del Llidoné; 8, Calsaes del Matà; 9, La Saltadora; 10, Coveta de Montegordo; 11, Cingle de la Eremita del Barranc Fondo; 12, Cingle dels Tolls del Puntal; 13, Cueva Grande del Puntal; 14, Covetes del Puntal.

The scene we are reporting is inside rock shelter no. 4, counting from the left, of the Cingle de la Eremita del Barranc Fondo, which is a small subsidiary of the Valltorta. This group of six rock shelters, of total length 96 m, is on the left bank of the narrow Barranc Fondo and can be reached only from the upper plateau. Rock shelter No. 4 contains several paintings in various shades of red, from a faded pinkish red to a reddish Sienna brown. In general the brownish paintings are the oldest. The panel is on the right side of the rear wall and undoubtedly depicts wild honey gathering activities (Fig. 2).

Human figures climbing ropes or ladders can be seen in several Levantine sites, but cannot always be interpreted as evidence for honey gathering. One well known scene is that of La Araña (Valencia) which has been reproduced many times[3]; a lesser known group is that of Dos Aguas (Valencia)[4], and we believe a third to be visible at Los Trepadores (Teruel)[6]. The new honey gathering scene would thus be the fourth.

The rock surface of the rear wall is uneven and shows many small fissures, crevices, and cup-like holes. The faded red colour of the painting indicates a late phase of Levantine art; this is confirmed by the small size of the human figures, as the tendency is for figures to become smaller and more schematic before the disappearance of this type of rock art. Several small cup-like holes at the top were the starting-point of the composition, for they depict the wild bees' nest. The similar scenes of La Araña and Dos Aguas also have a natural hole as a starting point. The total height of the scene, starting from the holes, is 52 cm.

Below the holes, two fissures have been made more conspicuous by outlining with red paint; at the right, the painted lines converge to a central stem evocative of the branch of a tree; on the left, the faded outline of a small animal, which may be a wild goat, can still be traced. Both fissures converge into the rough shape of a V; at this point, there is a squarish object with some appendages radiating out from the corners. This may represent a leather bag or basket for the gathering of honey. The actual ladder begins here and continues downwards for about 40 cm. The bees surrounding the ladder are disproportionately large, but this is also the case in the other honey gathering scenes.

The ladder is of complex construction, being the first of this type to be observed in Levantine rock art; it is a much more sophisticated version of the plain ropes attached to forked sticks at La Araña. At some places, sticks are attached to the side ropes, as can be seen on the segment of ladder trailing on the ground at lower left; at other places, the rope coils in a serpentine pattern. The thickening of the rungs at the point of contact with the ropes may indicate a knot, and the thickening of the side ropes may be a doubling or tripling of the rope at that particular point.

On the ladder, there are five human figures; the first two, at the top, are climbing in a doubled-up posture, grasping the ladder with both hands, and feet; the second figure from the top may be bearded. The third figure from the top seems to be jumping off or falling down; a fourth figure is again climbing up and the fifth, at bottom, is definitely jumping down from the ladder. A vertical fissure runs more or less parallel to the painted ladder.

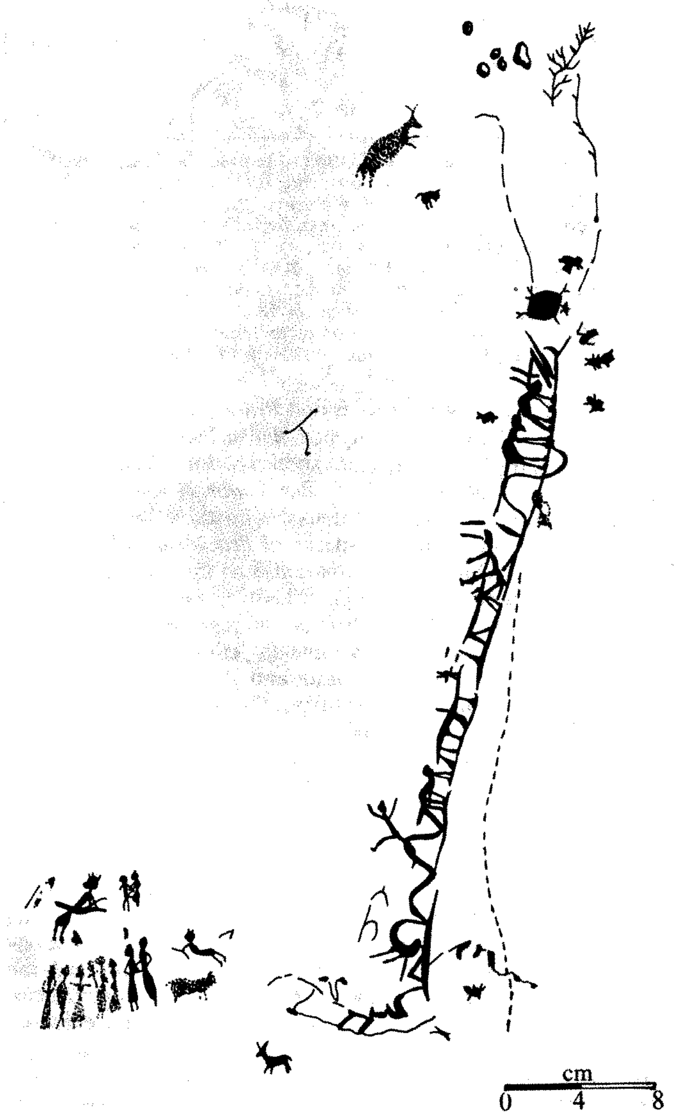

Fig. 2 The honey gathering scene from rock shelter no. 4 of the Cingle de la Eremita del Barranc Fondo.

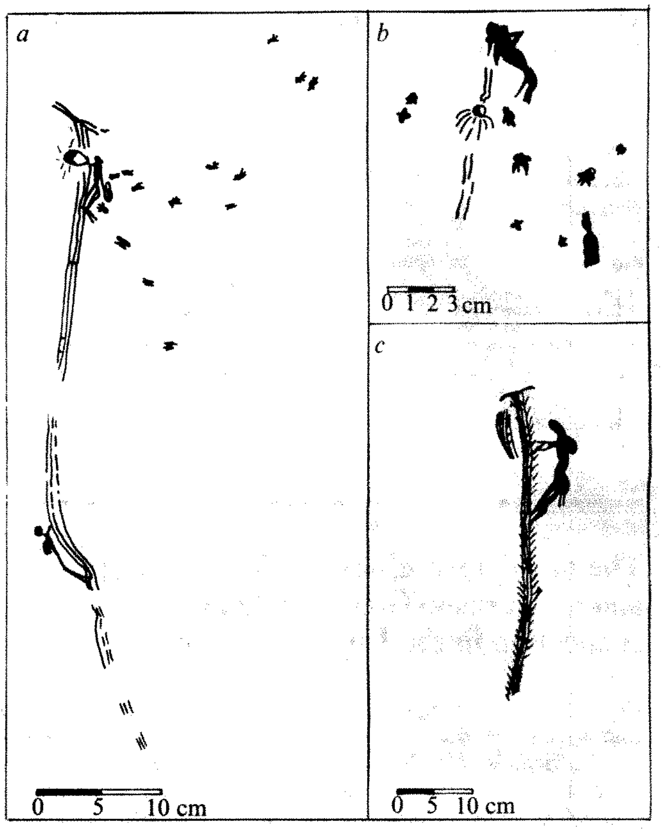

Fig. 3 The honey gathering scenes already known in Levantine rock art: *a*, La Araña; *b*, Dos Aguas; *c*, Los Trepadores.

At the bottom, there are 12 very small human figures, mainly female, a small animal and other remains; two male figures may have bows. The female figures do not seem to be engaged in any particular activity and have no ritual or ceremonial meaning, such as raised arms or dancing postures. This part of the scene is badly damaged by erosion and the small size of the figures (3–5 cm) does not make it easy to connect them with a honey-gathering activity, unless they are supposed to represent onlookers. This scene shows a much more advanced technology than those known from the same periods (Fig. 3), at La Araña (Valencia), Dos Aguas (Valencia) and Los Trepadores (Teruel), and it therefore throws new light on honey-gathering activities during the late Ice Age. Although the use of ropes is depicted in the other scenes, it is not known what type of fibres were involved. But the newly discovered scene shows a ladder built of ropes and wooden rungs, such as are still in use, mainly for mountaineering. The whole construction must have been very strong, as it supports the weight of five people (roughly 300–350 kg); this implies the use of adequately thick and straight sticks of similar weight for the rungs, and firmly tied knots to keep them in place. It would be interesting to know how the top of the whole contraption was attached: it was probably knotted around a projecting rock surface, near the rock fissure containing the wild bees' nest. It is not possible to speculate on the total length of the ladder, for the human figures are probably not drawn to scale.

Honey-gathering scenes are frequently depicted in South African rock art; in one example, at Anchor Shelter, Natal Drakensberg, the ladders are remarkably similar to that shown in the new scene[7,8]. In European rock art, however, the four scenes discussed here are the only indications of this type of activity among Late Stone Age hunters and food gatherers.

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The ornithopod dinosaur *Dryosaurus* and a Laurasia–Gondwanaland connection in the Upper Jurassic

COMPARISONS of Upper Jurassic ornithopod dinosaurs show that *Dysalotosaurus lettow-vorbecki* (Tendaguru Beds, Tanzania, East Africa) is referable to the genus *Dryosaurus* (Morrison Formation, western North America). This extended distribution of *Dryosaurus* provides additional evidence for a land connection between Laurasia and Gondwanaland sometime in the Upper Jurassic.

The close similarities of the Upper Jurassic dinosaur faunas of the Morrison Formation of the western United States and the Tendaguru Beds of Tanzania, East Africa (Tables 1, 2) are often cited as evidence for the presence of a Jurassic land connection between Laurasia and Gondwanaland (Fig. 1*a*). As regards genera common to both faunas (Table 1), most authors cite the enormous sauropod *Brachiosaurus*[1-5], and some also mention the smaller sauropod *Barosaurus*[1,2,6,7]. The complete anatomy of the Tendaguru species of *Brachiosaurus* is well known[7,8], but the material of *Barosaurus* is less complete[7]. The referral of the Tendaguru species to these genera is probably correct but as the type species of *Brachiosaurus* and *Barosaurus* from the Morrison Formation is so far each represented by only one partial skeleton described[9,10], the possibility that one or both of the Tendaguru species represent separate but related genera cannot be completely eliminated. The large carnivorous theropods *Allosaurus* and *Ceratosaurus* are represented by complete skeletons from the Morrison Formation, but the Tendaguru material tentatively referred[11] to these genera is extremely fragmentary. The one good skeleton of a medium sized theropod (*Elaphrosaurus*)[11] from the Tendaguru Beds is clearly distinct from the Morrison coelurids *Coelurus* and *Ornitholestes*. The stegosaurs or plated dinosaurs are well represented in both faunas, but *Stegosaurus* and *Kentrosaurus* are distinct and not closely related. The bipedal ornithopods are represented by four taxa from the Morrison Formation: the small fabrosaurid *Nanosaurus agilis*[12]; the hypsilophodontids *Othnielia rex*[13] (Table 1), and *Dryosaurus altus*; and the medium sized iguanodontid *Camptosaurus dispar*. Comparisons of the good material of *Dryosaurus altus* with the excellent material of *Dysalotosaurus lettow-vorbecki* (Fig. 2), the only Tendaguru ornithopod described[14], show that these taxa are congeneric as *Dryosaurus altus* (Marsh) and *Dryosaurus*

Table 1 Upper Jurassic dinosaur faunas from Morrison Formation of North America and Tendaguru Beds of East Africa

Saurischia: Theropoda	Morrison	Tendaguru
Coeluridae	*Coelurus*, *Ornitholestes*	*Elaphrosaurus*
Megalosauridae	*Ceratosaurus nasicornis* new genus*	*C.* (?) *roechlingi* *Megalosaurus* (?)
Allosauridae	*Allosaurus fragilis*	*A.* (?) *tendagurensis*
Incertae sedis	*Illosuchus*[29], *Marshosaurus*[30]	1 or 2 small species[11]
Sauropoda†		
Cetiosauridae	*Haplacanthosaurus*	
Brachiosauridae	*Brachiosaurus altithorax*	*B. brancai*
Camarosauridae	*Camarosaurus*	
Apatosauridae	*Apatosaurus*	
Diplodocidae	*Barosaurus lentus* *Diplodocus*	*B. africanus*
Dicraeosauridae		*Dicraeosaurus*
Titanosauridae		*Torniera*
Ornithischia: Ornithopoda		
Fabrosauridae	*Nanosaurus*	
Hypsilophodontidae	*Dryosaurus altus* *Othnielia*‡ n. g.	*D. lettow-vorbecki*
Iguanodontidae	*Camptosaurus*	
Stegosauria		
Stegosauridae	*Stegosaurus*	*Kentrosaurus*

Species given only when genus present in both faunas.

* To be described by author and J. A. Jensen of Brigham Young University, Provo, Utah.

† Families follow Janensch[7], valid Morrison genera following McIntosh and Berman[31].

‡ *Othnielia* n. g., named after Othniel Charles Marsh, type species *Nanosaurus rex* Marsh[32] (see ref. 13); type species of *Laosaurus* Marsh[33], *L. celer* Marsh[33], is a *nomen nudum* with *L. gracilis* Marsh[33] and *L. consors* Marsh[34] referable to *Othnielia rex* (Marsh).

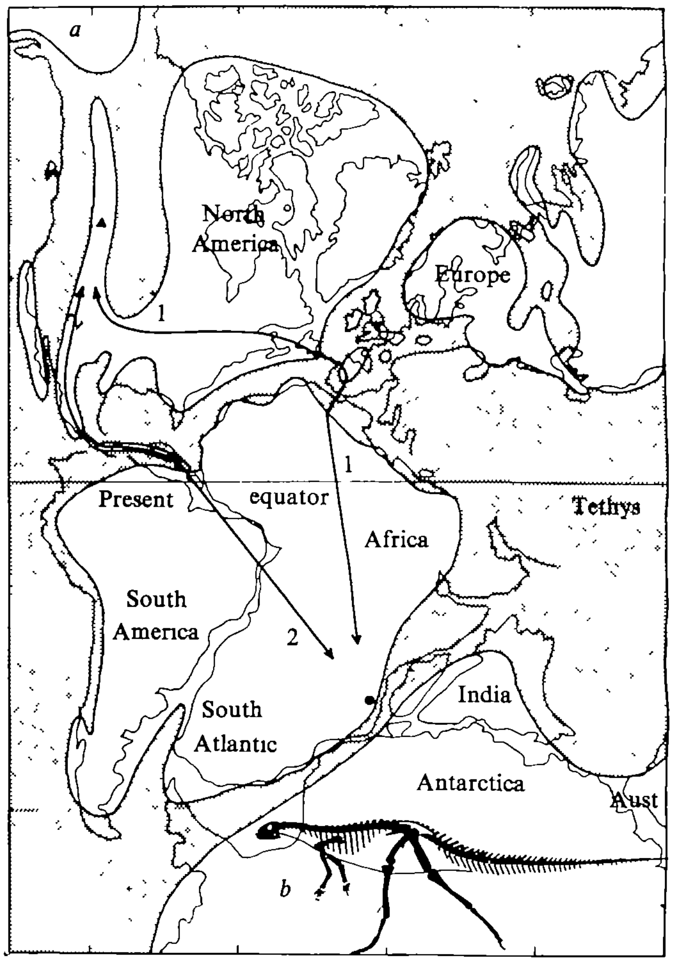

Fig. 1 *a*, Location and distribution of land in the western part of Laurasia and Gondwanaland during the Upper Jurassic (Kimmeridgian), after Ager[24]; —, reconstructed coast lines; —, present coast lines; ▲, *Dryosaurus altus* localities, western USA; ●, *Dryosaurus lettow-vorbecki* locality, Tanzania, East Africa; ▼, *Dryosaurus* sp. locality, England; Aust., Australia; 1, possible dispersal route via western Europe; 2, possible dispersal route via Central and South America. *b*, Skeletal reconstruction of *Dryosaurus lettow-vorbecki*, complete length of animal about 2.5 m, based on mounted skeleton HMN dy I[14].

lettow-vorbecki (Pompeckj). The osteology of these species of *Dryosaurus* is clearly distinct from that of the other hypsilophodontids *Othnielia*[12], *Hypsilophodon*[15] and *Parksosaurus*.

The skulls (Fig. 2*a*–*d*) show a few differences, but some of these (posterior region Fig. 2*a* and *c*, width Fig. 2*b* and *d*) result from crushing of the Morrison skull. In addition, two skulls[16] of *Hypsilophodon foxii* show greater differences in sutural pattern anteroventral to the orbit than do those of *Dryosaurus*. The main distinguishing character in the skull of *Dryosaurus altus* (Fig. 2*a*, *b*) is the more elongate supraorbital (Fig. 2*a*). The humeri (Fig. 2*i*–*l*) have low deltopectoral crests and low condyles distally (Fig. 2*i*, *k*, *l*). In the pelvic girdle (Fig. 2*m*–*r*), the main body of the ilium is low with an obliquely truncated posterior end, and the brevis shelf is broad; the distal half of the ischium is curved and bar-shaped with a proximally placed obturator process; and the anterior process of the pubis

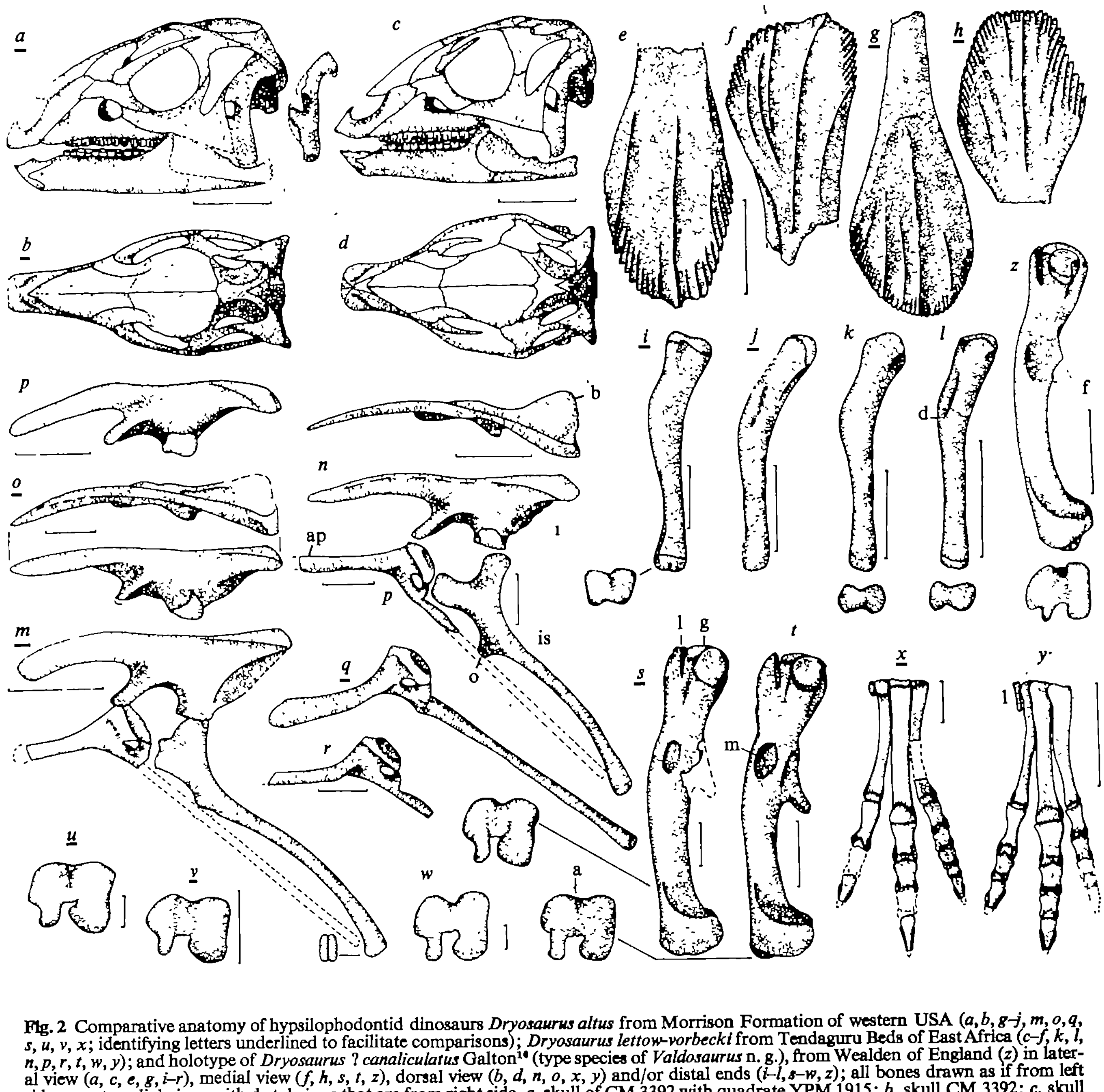

Fig. 2 Comparative anatomy of hypsilophodontid dinosaurs *Dryosaurus altus* from Morrison Formation of western USA (*a*, *b*, *g*–*j*, *m*, *o*, *q*, *s*, *u*, *v*, *x*; identifying letters underlined to facilitate comparisons); *Dryosaurus lettow-vorbecki* from Tendaguru Beds of East Africa (*c*–*f*, *k*, *l*, *n*, *p*, *r*, *t*, *w*, *y*); and holotype of *Dryosaurus ? canaliculatus* Galton[16] (type species of *Valdosaurus* n. g.), from Wealden of England (*z*) in lateral view (*a*, *c*, *e*, *g*, *i*–*r*), medial view (*f*, *h*, *s*, *t*, *z*), dorsal view (*b*, *d*, *n*, *o*, *x*, *y*) and/or distal ends (*i*–*l*, *s*–*w*, *z*); all bones drawn as if from left side except medial views with distal views that are from right side. *a*, skull of CM 3392 with quadrate YPM 1915; *b*, skull CM 3392; *c*, skull HMN dyA, modified after Janensch[14]; *d*, skull HMN dyA, modified after Janensch[14]; *e*, slightly worn maxillary tooth after Janensch[14]; *f*, moderately worn dentary tooth after Janensch[14]; *g*, unworn maxillary tooth YPM 1915; *h*, unworn dentary tooth YPM 1915; *i*, humerus YPM 1915; *j*, humerus AMNH 834; *k*, humerus UT 1495/22; *l*, humerus UT 1495/23; *m*, pelvic girdle AMNH 834; *n*, reconstruction of pelvic girdle (HMN dyI, dyII, dy35) with dorsal view of ilium (HMN dyII); *o*, ilium DNM 1016 in dorsal and lateral views; *p*, ilium UT 1495/9; *q*, pubis YPM 1884; *r*, pubis UT 1495/6; *s*, femur YPM 1915; *t*, femur HMN dy36; *u*, femur CM 21786; *v*, femur AMNH 834; *w*, femur UT 1495/14; *x*, pes CM 21786; *y*, pes HMN dyV; *z*, femur BMNH R185, after Galton[16]. *a*, Anterior intercondylar groove; ap, anterior process of pubis; b, brevis shelf; d, deltopectoral crest; f, fourth trochanter; g, greater trochanter; i, ilium; is, ischium; l, lesser trochanter; m, depression for M. caudi-femoralis longus; o, obturator process; p, pubis; l, vestigial first metatarsal. Scale lines represent 5 cm (*a*–*d*, *i*–*z*) or 1 cm (*e*–*h*). YPM 1915 is holotype of *Laosaurus altus* Marsh, the type species of *Dryosaurus* Marsh[34]. Specimens shown in *j*, *m*, *o*, *u*, *v* and *x* will be fully described elsewhere[35]. I thank the people indicated for their help while studying the figured specimens in their respective institutions indicated by the following abbreviations: AMNH, American Museum of Natural History, New York (E. S. Gaffney); BMNH, British Museum (Natural History), London (A. J. Charig, C. A. Walker); CM, Carnegie Museum, Pittsburgh (D. S. Berman); DNM, Dinosaur National Monument, Jensen, Utah (T. E. White, J. Adams); HMN, Humboldt Museum fur Naturkunde, East Berlin (W.-D. Heinrich, J. Helms, H. Jaeger); UT Universitat der Tubingen (F. Westphal); YPM, Peabody Museum Yale University, New Haven (J. H. Ostrom).

is slender with a variably developed ventrolateral edge. The femora (Fig. 2*s–w*) possess the same suite of characters: a deep cleft separating the lesser and greater trochanters; the deep depression level with the proximally placed fourth trochanter is set well anteriorly on the shaft; distally the medial edges are squared off, there is an anterior intercondyle groove, and the posterior lateral condyle is thin. In the pes the first metatarsal is rudimentary so there were only three movable toes (Fig. 2*x* and *y*).

Fragmentary evidence indicates the presence of *Dryosaurus* in Europe. A slender tibia (Sedgewick Museum, University of Cambridge specimen number J46889) from the lower Oxford Clay (Middle Jurassic, Middle Callovian) of Fletton near Peterborough, Northamptonshire (Fig. 1*a*) is probably referable to *Dryosaurus*. Femora (Fig. 2*z*) from the Wealden (Lower Cretaceous) of southern England that were tentatively referred[16] to *Dryosaurus* differ from femora of that genus in having a deeper anterior intercondylar groove, the anteromedial margin of which is squared off. A new genus *Valdosaurus* (see Fig. 2*z*) is erected for this material, which probably represents a Cretaceous descendant of *Dryosaurus*.

The presence of the hypsilophodontid dinosaur *Dryosaurus* in the Upper Jurassic (Upper Kimmeridgian, base of Tithonian)[1, 17] of western United States and Tanzania provides important additional evidence for the presence of a land connection between Laurasia and Gondwanaland (Fig. 1) sometime in the Upper Jurassic (Kimmeridgian or possibly Oxfordian). A North America–Europe–Africa route is usually assumed, with the connection located in southwest Europe and northern Africa[1–5] (Fig. 1). Support for this route is provided by the occurrence of *Apatosaurus* and *Brachiosaurus* in the Upper Kimmeridgian of Portugal[18]. But, Jurassic deposits in the critical region (southern Spain and Morocco) are largely marine[1,2,17] and, in addition, it is impossible to restore the coast lines in this area accurately as a result of the complexity of movements of the many small areas involved in the Alpine System orogeny[4, 19]. If a European route was involved then presumably a land bridge emerged from the shallow sea between Europe and North Africa for a brief period or periods in the Upper Jurassic.

Table 2 Comparisons between Morrison and Tendaguru dinosaur faunas

		Genera	Families
Morrison fauna (N or N_2)		18	14
Tendaguru fauna (N or N_1)		11	9
Morrison endemics (E)		9	0
Tendaguru endemics (E)		5	1
Taxa in common (C)		5	7
Simpson's coefficient $(C/N_1)\times 100$		45%	77%
Coefficient of difference $(1-C/N_2)\times 100$		72%	50%
Degree of endemism $(E/N)\times 100$	Morrison	50%	0%
	Tendaguru	45%	9%

No dinosaurs have yet been reported from the Jurassic deposits of Central America or northern South America[6], but the possibility that the Morrison–Tendaguru land connection involved the route North America–South America–Africa (Fig. 1) should not be ignored. The southern end of the South Atlantic opened first but a land route was still present between South America and Africa in the Lower Cretaceous (Aptian)[20]. The Central Atlantic began to open in the early Jurassic[1,19] with the Azores–Gibraltar ridge marking the line of shear between Europe and Africa as the latter separated from North America[1]. Gordon[21] shows a wide connection between the Pacific and the Central Atlantic throughout the Jurassic but his maps, like those in Colbert[5], are based on the reconstructions by Dietz and Holden[22]. In other reconstructions, North and South America are shown much closer together [1, 22-26] and even in contact[27]. Berggren and Hollister[23] consider that there was a marine connection for much of the Jurassic across Central America between the Pacific and the Central Atlantic, but note that this connection may have been intermittent during the Upper Jurassic. On the basis of marine invertebrates, Hallam[28] shows that a direct marine connection was not established until after the uppermost Middle Jurassic (Lower Callovian) and Ager[24] dates the connection as lower Upper Jurassic (Oxfordian)[24]. The maximum extent for Jurassic seas was reached during the Oxfordian and Kimmeridgian, when between a quarter and a third of the total continental area was covered[1]. While much of western Europe and northern Africa was submerged, there were proportionally shorter stretches of shallow sea separating North and South America[1,17,24-26] (Fig. 1) or possibly no sea at all[27].

The presence of *Dryosaurus* and other similarities (Tables 1, 2) in the Morrison and Tendaguru faunas clearly establishes the availability of a land connection between Laurasia and Gondwanaland sometime in the Upper Jurassic (Oxfordian?, Kimmeridgian) or possibly in the uppermost Middle Jurassic (Upper Callovian). But whether this connection was through Central America or western Europe cannot be determined at the moment.

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Vesicular–arbuscular mycorrhiza in some aquatic vascular plants

THE occurrence of vesicular–arbuscular (VA) mycorrhiza in flowering plants is a common phenomenon. Harley[1] pointed out that it is easier to list the few plants from which the endophyte is normally absent. It has been stated that aquatic plants are not infected by VA mycorrhiza[1,2].

Read *et al.*[3] found infection levels to be low and often absent in marsh plants and infection in *Cladium* spp. occurred only during dry periods[4]. During an investigation of root hair development in *Littorella uniflora, Lobelia dortmanna,* and *Isoetes lacustris* VA mycorrhiza was discovered in connection with evaluation of root uptake of inorganic carbon and nutrients[5]. Other submerged and a few emergent aquatic plants were also investigated.

Plants with intact root systems were collected in January and March 1977 in four softwater, oligotrophic lakes in central Jutland, Denmark. All samples were taken from 0.3–0.8 m depth to make sure that the plants had not at any time been above water level. The isoetid species were examined for development of root hairs by phase-contrast microscopy. The percentage of VA-infection was estimated by the root-slide technique[3]. Roots of randomly selected plants were cut into 1-cm segments, cleared and stained by the method of Phillips and Hayman[6]. All segments were examined for infection.

An *Endogone* sp. forming VA mycorrhiza was observed in five of the seven species examined (Table 1). Chlamydospores, vesicles and arbuscles were present in January but only vesicles and arbuscles were found in March. Of the isoetid species frequency of infection was highest in *Littorella uniflora* while *Lobelia dortmanna* was less infected. In agreement with earlier observations[1] no mycorrhiza was found in *Isoetes lacustris. Callitriche hamulata* was not infected in January, but intercellular vesicles were present in March. The type of vesicle was different from that observed in the other plant species. *Phragmites communis* and *Eleocharis palustris* were infected but the level of infection was low. At present nothing conclusive can be said about the differences of infection level of the same species in different lakes, but it is known that in terrestrial plants infection is heaviest in infertile soils[3].

Table 1 Percentage VA-infection in aquatic plants from four oligotrophic lakes, March 1977

Species	Kalgaard		Rævsø		Hampen		Almind	
	%	*N*	%	*N*	%	*N*	%	*N*
Littorella uniflora	82	44	22	79	36	76	96	92
Lobelia dortmanna	55	69	30	57			46	74
Isoetes lacustris	0	48	0	60	0	36		
Myriophyllum alterniflorum					0	43		
Callitriche hamulata					54	26		
Phragmites australis					+		+	
Eleocharis palustris					15	27		

N, Number of segments examined; +, infection observed.

Development of root hairs by the isoetid species is shown in Table 2. Root hairs were present in *Isoetes lacustris* but not in *Littorella uniflora* and *Lobelia dortmanna.* The observation by Shannon[7] that *Lobelia dortmanna* has root hairs could not be confirmed. The plants investigated by Shannon were seedlings cultivated in water, however, which may have influenced the development. In contrast our plants were mature and growing submerged in sandy sediments.

Table 2 Development of root hairs in three isoetid species

Species	Kalgaard	Rævsø	Hampen	Almind
Littorella uniflora	—	—	—	—
Lobelia dortmanna	—	—		—
Isoetis lacustris	+	+	+	

+, Root hair development present; —, development absent.

Ionic absorption and especially uptake of phosphorous is enhanced in plants infected by VA mycorrhizal fungi[8–10]. As the roots of aquatic vascular plants also function as organs for ionic absorption[2] the infection of VA mycorrhiza may have great influence on nutrient uptake, especially in *Littorella uniflora* and *Lobelia dortmanna* that are normally growing in sandy sediments poor in nutrients. The advantage of VA to these plants is further enhanced by the fact that the symbiotic system operates more effectively in low nutrient regimes[8]. The heavy infection of *Littorella uniflora* and *Lobelia dortmanna* is in agreement with the suggestion of Baylis[11] that plants with poorly developed root hairs may be obligatory mycotrophs in P-deficient soils. In contrast to *Littorella uniflora* and *Lobelia dortmanna, Myriophyllum alterniflorum* has its greatest absorptive surface in the free water and thus is less dependent on the root system which may explain the absence of mycorrhiza in this species.

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Evolution of long-distance alarm calls in Kloss's gibbon

We present evidence here that proximity of close kin is a plausible hypothesis to explain the evolution of long-distance alarm calls in Kloss's gibbon (*Hylobates klossii*). We discovered two alarm calls produced by adults of this monogamous ape that provide good locational cues and are audible for much greater distances than required to signal the immediate family. Our further finding that at least some Kloss's gibbons settle in territories adjacent to their parental families when they mature and establish their own reproductive groups suggests that the adaptive significance of these calls is to warn neighbouring relatives of the presence and location of danger.

Many species of birds and mammals, including primates, emit vocal alarm signals when a potential predator is detected[1,2]. Alarm calls of birds in particular have received much attention because they well illustrate the adaptive significance of signal structure[3] and because they have been interpreted as animal altruism evolved by kin selection[4]. Interpretation of alarm calls relative to kin selection theory, however, has been impaired by the paucity of data on the distribution of related individuals in wild populations. It is confined further by the lack of knowledge on how predators respond to alarm calls of their prey[1]. These difficulties are partially overcome in this study of alarm calls given by Kloss's gibbons.

Kloss's gibbon is strictly endemic to the four Mentawai Islands, 85–145 km off the west coast of Sumatra, Indonesia[5]. This study was conducted in evergreen rain forest on Siberut, the northern-most Mentawai Island, from September 1971 to October 1974, particularly during 387 d at Teitei Peleigei Forest (1°24′S, 99°1′E) where we identified 15 gibbon groups and their territories. Kloss's gibbons live in monogamous families of two to six individuals and defend territories of 7–11 hectares (ref. 5 and R.L.T. in preparation).

Ten kinds of Kloss's gibbon vocalisations have been identified (ref. 6 and Table 1). Two of them, produced by adults of both sexes, are long-distance alarm calls referred to as sirening and alarm trills (Fig. 1). They were audible to us on the ground up to 800 m away (based on marked, tape-measured trails) and indicate specifically the detection of a potential predator. Audibility range of gibbon calls to a man on the ground may be different from that to another gibbon, up in the forest canopy. It also may vary with time of day[7] and local atmospheric conditions (K. Henwood and A. Fabrick, in preparation). Nonetheless, our ground-level determinations are useful to illustrate relative audibility of various calls in the Kloss' gibbon repertoire. They show that the only vocalisations with audibility ranges as great as those of sirening and alarm trills are male and female songs (Table 1), which apparently function in maintenance of territorial organisation among neighbouring groups[8].

We noted 38 bouts of sirening and alarm trills during our study at Tei-tei Peleigei Forest, 21 times from gibbon families disturbed by one of us and 12 times in response to native hunters. Cause in the other five cases was undetermined, although hunters may have been involved. Sirening and alarm trills that we heard during surveys elsewhere in Siberut in 1971 were also in response to human disturbance. Man is the major and perhaps only stimulus eliciting these long-distance alarm calls. This is appropriate since Siberut natives depend heavily on primate flesh for protein, and there are no other predators on Kloss's gibbon except possibly the reticulated python[5]. The hunters, armed only with a palmwood bow and unfletched poisoned arrows, depend on stealth to approach their prey. Despite several hundred years or more of being hunted by Siberut natives, the Kloss's gibbon population is denser than any other hylobatid population known[5].

The repetitive, frequency-modulated syllables of sirening, the rapidly repeated elements in the alarm trill, and the rich harmonic structures of both (Fig. 1), are characteristics which facilitate directional location of a signaller by recipients making binaural comparisons of phase, time and intensity[1,3]. Our own experience at distances to 800 m is that sirening, which normally (>80% of instances) begins before alarm trills, sounds the general alarm that a predator is in a specified area. Subsequent alarm trills pinpoint its location, providing more accurate information about direction and distance. Redundancy of the calls provides recipients ample opportunity to obtain this information: during tape-recorded bouts of four alarm-calling gibbon pairs, sirening occurred at an average rate of once every 95 s per pair and alarm trills once every 19 s per pair. Thus an average bout of 9.4 min (s.d. ± 7.5 min, R = 2–27, N = 21) includes about 30 alarm trills and 6 sirening calls.

Table 1 Estimated audible ranges of adult Kloss's gibbon vocalisations to a man on the ground

	Vocalisation	Context	Estimated audible range (m)
1	Hoo	Detection of langurs, other gibbons, or humans	50–100
2	Howl	Detection of langurs, other gibbons, or humans	100–150
3	Whup	During female song bouts. Occasionally precedes male song, sometimes emitted on detecting humans	400
4	Whistle	Precedes male song; occurs during female song bouts and is occasionally emitted upon detecting humans	50–100
5	Whoo	Precedes female song	400
6	Sirening	Detection of a human	800
7	Alarm trills	Detection of a human	800
8*a*	Male song	Begins spontaneously or on hearing other males singing	500–700
8*b*	Female song	Begins spontaneously, on hearing other females singing or on encountering another female at territorial boundary	800
9	Quivering squeal	Males fighting (emitted by males)	50–100
10	Whistle–howl	Males fighting (emitted by females)	50–100

Note that six of the ten call types may occur when a predator (human) is detected but only sirening and alarm trills are restricted to this context.

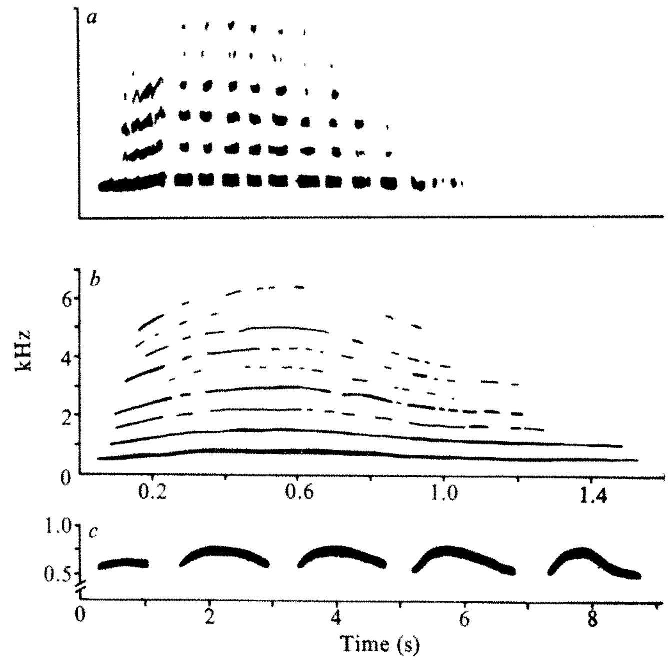

Fig. 1 Ink tracings of audiospectrograms of Kloss's gibbon loud alarm calls. *a*, Alarm trill (time and frequency as in *b*). *b*, One syllable of a sirening call to show harmonic structures. *c*, Example of a complete seven-syllable sirening call (harmonics were deleted in producing the spectrogram, only fundamentals are shown). Mean number of syllables per sirening call is 4.8 (s.d. ± 1.4, R = 3–7, N = 11) and mean duration of the call is 7.8 s (s.d. ± 2.4, R = 5–12, N = 11). Mean duration of the alarm trill is 1.0 s (s.d. ± 0.1, R = 0.93–1.28, N = 21). In 75% (39/52) of cases the alarm trill is preceded by one to three loud 'whups'. Tenaza recorded these vocalisations in the field with a Uher 4400 tape recorder (run at 3 3/4 inches per second) and a Sennheiser 405-S microphone fitted to an aluminium parabola 60 cm in diameter. Audiospectrograms were produced with a Kay Elemetrics sound spectrograph. *a* and *c* were made on wide band setting, *b* on narrow band.

Aboriginal hunters attempting to approach undetected to within bow and arrow range (50 m or less) of Kloss's gibbons usually abandon the effort if the gibbons discover them, although they normally pursue the other primates (*Presbytis potenziani*, *Simias concolor*, and *Macaca pagensis*) in similar circumstances. Hunters give two reasons for this, first alerted gibbons are unusually difficult targets because of their rapid movement in the upper reaches of the canopy, and second, the gibbons' loud alarm calls alert other primates in the vicinity which lowers the hunter's chances of success. We observed all four species of anthropoid primates in Siberut confronting hunters but only the gibbon produced predator-specific alarm calls audible to such great distances. Although the Mentawai langur (*P. potenziani*) emits an alarm call audible to about 500 m, it is given when confronting conspecifics and gibbons as well as when encountering humans[9].

Apparently because a Kloss's gibbon does not further endanger itself by revealing its position to the predator (human hunters), signals that not only announce the presence of danger but also inform recipients of its precise location have been allowed to evolve. If hunters were more capable of capturing alerted gibbons, selection might have resulted instead in alarm calls that are difficult to locate, like the ventriloquial whistles certain passerines and rodents emit when a predator such as a flying raptor is detected[3,10,11].

Sirening and alarm trills may have evolved simply to warn mates and offspring. But, members of a Kloss's gibbon family rarely are more than 10 m apart[5], whereas sirening and alarm trills are audible for about 800 m, more than twice the diameter of an average territory (x diameter = 350 m). Since softer alarm calls in the Kloss's gibbon vocal repertoire (Table 1) are sufficient to signal the immediate family, we suggest their loud alarm calls evolved by selection favouring gibbons who warn neighbours of danger.

Evidence on gibbon genealogy indicates a high probability that gibbons occupying neighbouring territories are close relatives. Four instances of settling by Kloss's gibbons in Siberut were observed. In 1972 a male was apparently being aided by his parents to establish a territory contiguous with theirs, and a female consorted[5] and finally mated (R.L.T., in preparation) with a male holding a territory contiguous to that of her parents. Two additional cases of settling were documented in 1973–74. In one a male was aided by his parents to establish a territory situated 150 m from theirs, and in another a female was accompanied by her parents while being courted by a male whom she eventually settled with in a territory contiguous with that of her parents (R.L.T., in preparation). In each of the three cases where a successful breeding pair was formed, at least one of the pair was the offspring of the occupants of an adjacent or nearby territory. While spatial limitations and the presence of floating males in the population[5] indicate that not all gibbons settle within audible range of their parental families, these observations show that at least some do. In the only other case of settling by a wild gibbon which has been observed, Aldrich-Blake and Chivers[12] report that a male siamang (*H. syndactylus*) established a territory contiguous with that of his parents on reaching physical maturity. Although siamangs do not have specialised long-distance alarm calls, they do emit a distinguishable rendition of their loud territorial call when alarmed by a potential predator[13].

In each case cited above a mature offspring settled within audible range (for long-distance alarm calls) of parents and younger siblings, with each of whom it shares a coefficient of relationship of 0.5. Theoretically, in such a socio-genealogical framework, long-distance alarm calls that do not appreciably lower the individual fitness of a signaller could increase the inclusive fitness of neighbouring relatives and thus be favoured by kin selection[4,14,15]. The fact that every species of gibbon which has been studied produces some kind of distinguishable long-distance alarm call[16–18] suggests this could be true for all species of hylobatids. The apparently high probability of close genetic relatedness among neighbouring Kloss's gibbons makes it unnecessary, in our opinion, to invoke hypotheses of reciprocal altruism[19] or selfish manipulation[20,21] in attempts to explain the evolution of alarm calls in this species.

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Role of sister chromatid exchanges in chromatid aberration formation

MECHANISMS of formation of chromatid aberrations are not fully understood. The involvement of chromosome breakage and reunion[1] or exchange[2] has been suggested. DNA repair is a possible cause of aberrations and exchanges[3–5], but a causal relationship between the two phenomena is not clear[6,7]. It is not known whether chromatid deletions derive from unrepaired chromatid breaks or from incomplete exchanges between sister chromatids. The origin of chromatid deletions can be studied by examining whether or not they occur just at the points of sister chromatid exchanges (SCE) (ref. 6). Differential labelling of sister chromatids with 5-bromodeoxyuridine (BUdR) has shown that X-ray-induced chromatid deletions are only infrequently associated with SCE, indicating that most chromatid deletions are not incomplete SCE but simple chromatid breakage[8]. On the other hand, the parallel distribution of SCE and chromosome aberrations induced by two different hydrocarbon carcinogens, 7,12-dimethylbenz (*a*) anthracene and 7,8,12-trimethylbenz (*a*) anthracene has been reported, suggesting that aberrations are incomplete SCE (ref. 9). The results presented here indicate that almost no chromatid deletions or gaps induced by irradiation of BUdR-substituted chromosomes with visible light represent incomplete SCE.

When Chinese hamster ovary (CHO) cells cultured for two rounds of replication in the presence of BUdR were

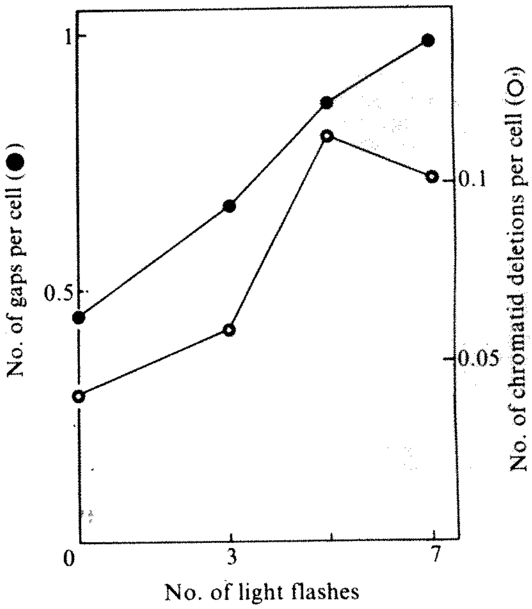

Fig. 1 Frequencies of chromatid deletions and gaps plotted against the number of light flashes. CHO cells were cultured in the dark in McCoy's 5A medium containing 15% foetal calf serum and 10 μM BUdR for 24 h. The cells were then exposed to visible light from an electric photographic flash unit placed 10 cm from the cell layer: the light was filtered through a 5-mm acryl–resin plate. Colcemid (final concentration 10^{-7} M) was added to the culture 2 h before collecting the metaphase cells. The sample was therefore irradiated with visible light in the G_2 phase. Chromosome specimens were prepared and the slides were stained by the Giemsa[12,13] or modified 33258 Hoechst–Giemsa methods[14]. 100 cells were observed for each light intensity.

exposed to visible light from a photographic electric flash unit, chromatid aberrations, excluding exchange types, and SCE, were induced frequently. Using such cells, we investigated whether or not chromatid deletions occurred just at the points of SCE.

The frequencies of chromatid deletions and gaps increased with increasing light flashes, or intensity of visible light (Fig. 1). The number of SCE in each chromosome was also in proportion to the light intensity and the relative size of chromosomes (Fig. 2) (ref. 12). Chromatid aberrations and SCE observed may have resulted from DNA strand breaks induced by photolysis of BUdR-containing chromosomes, for photolysis of BUdR-incorporated DNA leads to polynucleotide strand breaks[10].

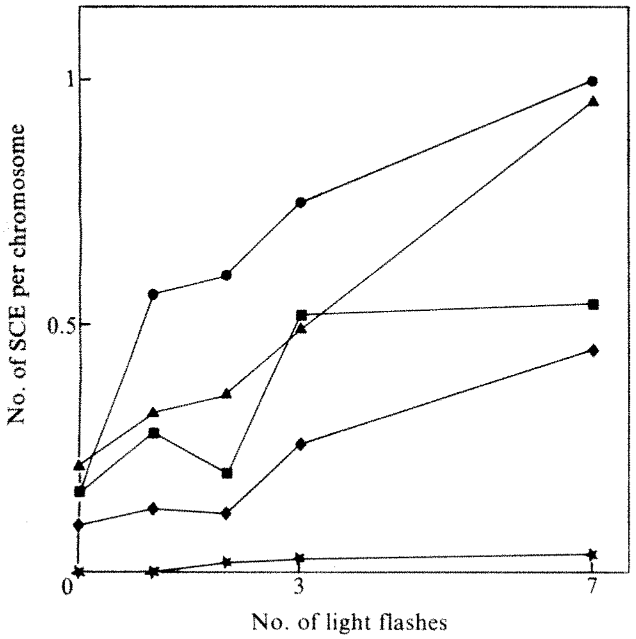

Fig. 2 Frequency of SCE plotted against the number of light flashes. SCE were scored for the largest (●), the second largest (▲), the third largest (■), medium-sized (◆) and small (★) chromosomes, respectively. Each data point represents the analysis in more than 25 chromosomes.

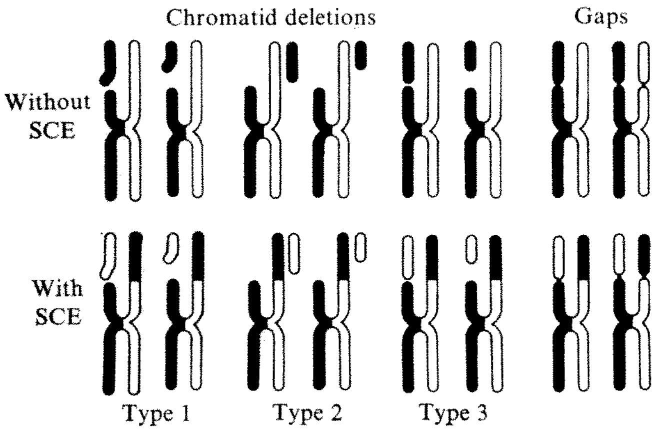

Fig. 3 Schematic representation of three types of chromatid deletions and gaps scored. Type 1, slightly displaced distal segment; type 2, completely displaced distal segment; type 3, nondisplaced distal segment.

Table 1 Chromatid deletions and gaps with or without SCE at breakpoints

Chromatid type*	Chromatid deletions†								Gaps	
	Type 1		Type 2		Type 3		Total			
	+	−	+	−	+	−	+	−	+	−
BB	0	1	0	4	1	24	1	29	22	440
BT		1		2		9		12		170
Total	0	2	0	6	1	33	1	41	22	610

+, With SCE; −, without SCE.
* BB, BUdR bifilarly substituted; BT, BUdR unifilarly substituted.
† Classified according to Fig. 3.

Chromatid aberrations were scored according to whether aberrations were accompanied by SCE. Chromatid deletions were classified into three types according to the position of distal segments (Fig. 3). Only one out of 42 chromatid deletions and 22 out of 632 gaps observed contained SCE at the breakpoints (Table 1). These results indicate that very few chromatid deletions or gaps are incomplete exchanges. This is consistent with the observations for X-ray-induced chromatid deletions[8]. The lesions and/or subsequent processes leading to chromosome aberrations might be different from those involved in sister chromatid exchanges.

Table 1 also implies that bifilarly BUdR-substituted chromatids are more than twice as sensitive to light flashes as are unifilarly substituted chromatids. Equal sensitivity has been found to X rays in unifilarly- and bifilarly-substituted chromosomes, both of which are more sensitive than unsubstituted chromosomes[8,11]. This apparent difference in sensitivity might reflect some fundamental distinctions between the two types of radiation for production and repair processes of DNA strand breaks responsible for aberrations in BUdR-containing chromosomes, although it is difficult to speculate on the nature of the distinction.

The results presented here indicate that, whatever the mechanisms of formation of chromatid aberrations or SCE, the relationship of SCE to chromatid breaks depends on the type of agent used to damage the chromosomes.

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Interferon suppresses the transition of quiescent 3T3 cells to a growing state

PAUCKER, Cantell and Henle reported that crude mouse interferon preparations depressed multiplication of suspended L cells[1]. Since then evidence has accumulated indicating that interferon molecules which inhibit multiplication of virus also inhibit the growth of cells in culture[2–9]. Interferon was shown to suppress incorporation of ^{3}H-thymidine into cellular DNA in various systems—for example, release from a double thymidine block in synchronised L 929 cells[10]; activation of mouse spleen lymphocytes by phytohaemagglutinin (PHA) or allogeneic lymphocytes[11]; and in steady-state growth of cultured mouse leukaemia L 1210 cells[12] in a chemostat. However, there has been no direct evidence to distinguish between the effect of interferon on the rate of initiation of DNA synthesis (entry into the S phase) and that on the rate of DNA synthesis in the S phase, and it is uncertain which step of the cell cycle is interfered by interferon. It is well known that most cells in cultures of confluent or serum starved mouse 3T3 stay in the early G_1 or G_0 stage of the cell cycle, and an addition of serum to these quiescent cells stimulates their growth, leading to DNA synthesis and cell division[13,14]. We describe here a suppressive effect of interferon on the transition from the quiescent to the growing state in BALB/c 3T3 cells. The data indicate that interferon suppresses the initiation of DNA synthesis.

Interferon preparations used were obtained from the supernatant of monolayer culture of mouse L cells inoculated with Newcastle disease virus and purified by column chromatography using DEAE–Sephadex and CM-Sephadex (2×10^7 units per mg protein) (ref. 15). Interferon titres were expressed in international mouse reference units.

Confluent and serum-starved quiescent BALB/c 3T3 cells were transferred to fresh medium containing 10% foetal bovine serum and ^{3}H-thymidine with or without interferon, and the proportion of cells with labelled nuclei (labelling index) was determined as a function of incubation time by autoradiography (Fig. 1*a*). The labelling index shows the proportion of cells which have entered the S phase. The cells synthesising DNA began to appear after a 12-h lag period either in the presence or absence of interferon, but the rate of increase of the DNA-synthesising cells was reduced when interferon was present in the culture; that is, interferon reduced the frequency of the initiation of DNA synthesis, but it did not shift the point where cells began to enter into the S phase. The result is reminiscent of the fact that, although serum concentration determines the rate at which cells enter the S phase, the length of the lag period is independent of the serum concentration[17].

Smith and Martin have proposed a new theory on the cell cycle, in which a cell's life is divided into two fundamentally different parts: A-state and B-phase[18]. The B-phase is a determinate one including the S, G_2 and M phases in addition to a part of the G_1 phase. On the other hand, the A-state is rather indeterminate. After mitosis the cell enters the A-state and remains for any length of time with a constant probability of leaving this state ('transition probability'). Brooks has analysed the regulation of the fibroblast cell cycle by serum according to the above theory, and showed that serum factors control the rate of transition from the A-state to the B-phase[17,19].

Figure 1*b* replotted from Fig. 1*a* shows the proportion of cells remaining in the G_1 phase (cells unlabelled) indicating that after 12-h lag period the rate of decrease of the G_1 cells either in the presence or absence of interferon followed first-order kinetics. In this experiment the transition rate constant was 0.13 h^{-1} in interferon-free control cultures, while in those with added interferon (1×10^4 units per ml) it was 0.036 h^{-1}. The rate constant estimated here is not exactly the same as 'transition probability' (P) defined by Smith and Martin[18], since in our experiments a small portion of the quiescent 3T3 cells was not in the G_0 or G_1 phase (A-state). It is closely related to P, however, because the rate of decrease of unlabelled cells after a lag period followed first-order kinetics. Thus, it can be said that interferon reduced the 'transition probability' from the A-state to the B-phase. Furthermore, the transition rate constant declined proportionally to the amount of interferon added (Fig. 2).

The effect of interferon on the transition from the G_1 phase to the S phase was examined when interferon was added at various times following serum stimulation (Fig. 3). When added at zero time or 4 h, the suppressive effect of interferon on the transition was almost the same, but after 4 h it declined, and at the time when the cells began to enter into the S phase the addition of interferon did not show any effect on the initiation of DNA synthesis. A certain period may be required for the expression of the suppressive activity of interferon, or interferon may not have any effect on the cells already committed to DNA synthesis.

Although our interferon preparations are not completely pure, the following results strongly indicate that interferon itself is responsible for the observed effect. First, when the interferon preparations were heated at 100 °C, their antiviral

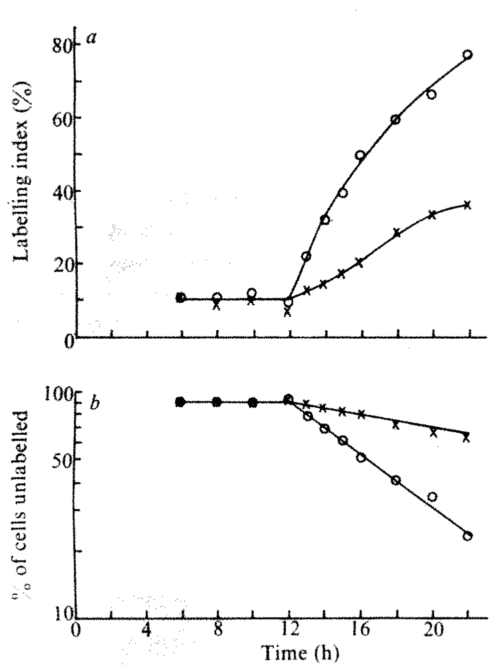

Fig. 1 Effect of interferon on the entry into the S phase after stimulation of quiescent BALB/c 3T3 cells with addition of fresh medium containing 10% serum. BALB/c 3T3 cells[16] (from Flow Laboratories) were plated at 4×10^4 cells on to 15-mm plastic coverslips (Wako Pure Chemical Industries) submerged in each well of Linbro multi-dish tray (Linbro 24-16-TC) containing 1 ml of Dulbecco's Modified Eagle's Medium (GIBCO) with 10% foetal bovine serum (Microbiological Associates), and grown at 37 °C in a 10% CO_2 atmosphere (v/v) until confluent. Four days after they were seeded, cell monolayers ($\sim 2 \times 10^5$ cells per plate) were washed with phosphate-buffered saline, and added 0.5 ml of serum-free Modified Eagle's Medium. After incubation for further 12 h, the medium was discarded and replaced by 0.5 ml of fresh medium containing 10% foetal bovine serum and ^{3}H-thymidine (1 μCi ml^{-1}; 2 μM thymidine) with or without addition of interferon (1×10^4 units per ml). At the times indicated, coverslips were removed and cells were washed with phosphate-buffered saline and extracted with 5% perchloric acid, rinsed with ethanol and air dried. Coverslips were mounted on to glass microscope slides and they were dipped into SAKURA autoradiographic emulsion NR-M2 (diluted with an equal volume of distilled water). After exposure for 7 d at 4 °C, autoradiographs were developed, stained with Giemsa solution, and the proportion of cells with labelled nuclei was determined. *a*, Proportion of cells entering into the S phase (labelling index). *b*, Proportion of cells remaining in the G_1 phase (100 minus labelling index). ○, No interferon; ×, Interferon added.

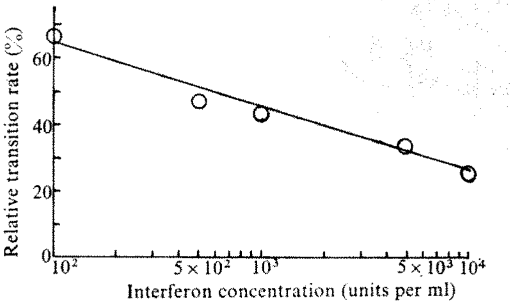

Fig. 2 Relative transition rate as a function of interferon dose. Quiescent and serum starved BALB/c 3T3 cells were stimulated by the addition of fresh medium containing 10% foetal bovine serum as described in Fig. 1. Various doses of interferon were added at zero time. Coverslips were removed at 8, 10, 12, 14, 16, 18, 21 and 24 h, respectively, and processed by autoradiography. Autoradiographs were exposed for 12 d. The proportion of cells remaining in the G_1 phase was plotted semilogarithmically as a function of time and the transition rate constant was obtained. In interferon-free control cultures it was 0.10 h^{-1}. Ordinate indicates the relative transition rate to the control.

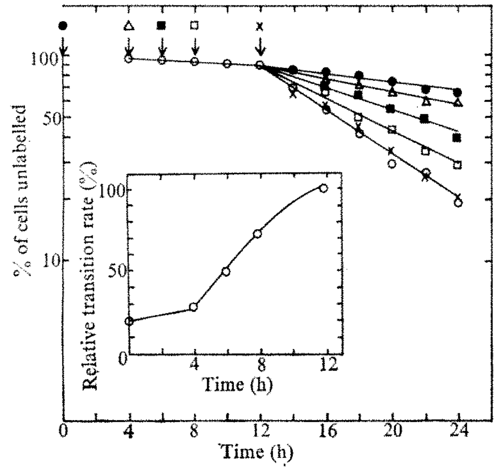

Fig. 3 Effect of addition of interferon at various times during stimulation on the rate of entry into the S phase. Quiescent and serum-starved BALB/c 3T3 cells were stimulated by the addition of fresh medium containing 10% foetal bovine serum, and interferon (1×10^4 units per ml) was added at the times indicated. Coverslips were removed and processed by autoradiography. Autoradiographs were exposed for 7 d. The inset to the figure describes the relative transition rate as a function of the time when interferon was added. In interferon-free control cultures the transition rate constant was 0.13 h^{-1}.

activity was reduced to less than 1%, but on heating in the presence of sodium dodecyl sulphate (SDS), about one-third of it survived (Table 1). The suppressive effect of interferon on the transition from the quiescent to the growing state of the cells was also lost by heating, but preserved if heated in the presence of SDS, in parallel to the antiviral activity (see the dose–response curve in Fig. 2). Similar results have already been reported by Stewart *et al.*[9]. Second, both activities were lost on trypsin digestion of the interferon preparations (Table 1). Third, the L cell interferon preparations, used at 1×10^4 units per ml, did not exert any effect on the serum activation of the serum-starved quiescent BHK cells (data not shown), conforming to the well-known species specificity of interferon action.

Interferon molecules may suppress certain cellular events in the G_1 stage of the cells, since the values for the transition rate constant ('transition probability') reflect the cellular activities during the transition from a quiescent to a growing state (G_1 phase or A-state)[19,20]. The transition is known to be accompanied with a complex set of cellular changes (see ref. 21), termed the "pleiotypic control" by Hershko *et al.*[22]. It was reported that interferon elevates the cellular levels of cyclic AMP in the cultures of L 929 cells[23]. The suppressive effect of interferon on the initiation of DNA synthesis may be resulted partly from the failure in falling of cyclic AMP levels. We have recently observed the effect of interferon on the growth rate, labelling index, fraction of labelled mitosis and mitotic index of BALB/c 3T3 cells in exponentially growing culture. The data showed that the duration of the G_1 phase was only affected by the addition of interferon (in preparation).

Table 1 Antiviral and anticellular activities of interferon after heating with or without SDS, and trypsin digestion

Treatment of interferon*	Antiviral activity† survival (%)	Anticellular activity‡	
		Transition rate constant (h^{-1})	Relative transition rate (%)
No treatment	100	0.015	20
−SDS, 100 °C, 1 min	0.5	0.038	51
+SDS, 100 °C, 1 min	34	0.013	18
−SDS, 100 °C, 4 min	0.1	0.043	58
+SDS, 100 °C, 4 min	34	0.013	18
+Trypsin, 37 °C, 30 min	0.04	0.051	69
Without interferon	—	0.074	100

*Small tubes containing 20 µl of L cell interferon (1×10^7 units ml^{-1}, 0.5 mg protein ml^{-1}) in phosphate-buffered saline were prepared. They were constituted to contain 1% SDS–0.1 mM dithiothreitol or 0.1% trypsin (Sigma) when indicated. After treatment, 80 µl of Dulbecco's Modified Eagle's Medium containing 10% foetal bovine serum was added.

†Antiviral activity was assayed by the method described by Suzuki *et al.*[24]. Reduction of incorporation of ^3H-uridine into RNA of vesicular stomatitis virus in actinomycin-D-treated mouse L cells was estimated.

‡Effect of interferon on the transition from a quiescent to a growing state in BALB/c 3T3 cells was observed as described in Fig. 1. Non-treated interferon was used at 5×10^4 units per ml.

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Inhibition of lymphocyte-induced angiogenesis by isolated chondrocytes

LYMPHOCYTE-induced angiogenesis (LIA) has been introduced by Sidky and Auerbach[1] as a quantitative and sensitive assay for measuring cell-mediated immunity *in vivo*. In this assay immunocompetent lymphocytes are injected intradermally into allogeneic or semiallogeneic mice. A local graft versus host (GVH) reaction is therefore induced, resulting in new blood vessel formation. As early as the second day post-injection, this effect could be observed in a dose–response fashion dependent on the number of cells injected. The relationship of lymphocyte-induced angiogenesis to tumour-induced angiogenesis mediated by tumour-angiogenesis factor (TAF)[2] remains theoretical. Folkman and others have demonstrated inhibition of TAF-induced angiogenesis by cartilage fragments[3] or extracts[4]. In this study we show that isolated chondrocytes can inhibit the angiogenesis induced by lymphocytes *in vivo*. Their ability to do so depends on the ratio of chondrocytes to lymphocytes used.

LIA assays were performed in 8–10-week-old female (CFW × C57Bl/Sn)F_1 hybrid mice. Recipients were injected intradermally with 0.1 ml of Hanks solution containing from 1.0×10^6–4.0×10^6 viable lymphoid cells obtained from 8–10-week-old parental CFW female donors by mechanical disruption of the spleen in a loose-fitting glass homogeniser. Control injections contained 4×10^6 viable syngeneic F_1 spleen cells or 4×10^6 parental CFW thymocytes.

Chondrocytes were obtained from cartilaginous epiphyses of long bones dissected from 6–10-d-old CFW mice. Cartilage fragments were digested with a mixture of 0.25% collagenase, 0.25% trypsin and 0.05% streptodornase in Hanks solution[5]. Cells liberated from the matrix were rinsed three times in Hanks solution with 30% calf serum and then twice in Hanks solution alone. Viability, estimated with the Trypan blue exclusion test, was greater than 85%.

To measure the effect of chondrocytes on lymphocyte-induced angiogenesis, variable numbers of viable chondrocytes (from 0.2×10^6 to 2.0×10^6) were mixed with 4×10^6 viable syngeneic or parental spleen cells in a total volume of 0.1 ml of Hanks solution to obtain a chondrocyte–lymphocyte ratio of 1 : 20, 1 : 10, 1 : 5 or 1 : 2 respectively. Control injections consisted of chondrocytes only or of spleen cells mixed either with freeze–thaw killed chondrocytes or with viable kidney cells obtained from adult CFW donors by digestion with the enzymatic solution used for chondrocyte isolation. Trypan blue (2–3 drops) was added per 1 ml of each inoculum injected to facilitate the subsequent location of the injection site. To eliminate the possibility of individual recipient variations in reactivity, each animal received up to 8 different inocula, tested in a coded manner. The animals were killed after 3 d and the skin carefully dissected from the underlying muscles to expose its inner surface under a dissecting microscope using a green filter. All blood vessels directed towards the injection site and contrasting with the background vasculature because of their contorted shape and pathways were scored according to the criteria proposed by Sidky and Auerbach[1]. The number of vessels evoked by intradermal injection of parental spleen cells compared with other cell types is shown in Table 1.

The lymphocyte-induced angiogenesis assay used in the present study is a lymphocyte-dependent at least initially, when there is lymphocyte contact with the host antigens. Specific recognition by competent cells must first take place to induce neovascularisation, since the angiogenesis evoked by syngeneic F_1 spleen cells is negligible. Also, angiogenesis induced by parental thymocytes is weaker than that induced by parental spleen cells. Lymphocytes stimulated by host antigens may release angiogenetic factors or, alternatively, these factors could be products of tissue destruction resulting from an immunological reaction. Lymphocytes also produce substances chemotactic for granulocytes[6,7] and polymorphonuclear leukocytes have been shown to evoke angiogenesis in the rabbit cornea[8]. However elicited, the stimulatory effect on capillary growth is related to the number of the competent lymphocytes injected.

These results show that chondrocytes, in appropriate concentration, inhibit the angiogenesis induced by lymphoid cells. At a chondrocyte–lymphocyte ratio of 1 : 10 this effect is most marked, reducing the intensity of the angiogenetic response evoked by 4×10^6 spleen cells to a level similar to that produced by 2×10^6 spleen cells. This effect is specific for viable chondrocytes since neither killed chondrocytes nor kidney cells show this inhibitory effect on angiogenesis.

Isolated chondrocytes produce matrix when injected into recipient animals[5] and some of their products could affect endothelial cell growth. Small cationic proteins with protease-inhibiting properties have been shown to be responsible for the resistance of cartilage to invasion by vascular mesenchymal cells[9,10]. Cartilage extract inhibits the growth of endothelial cells *in vitro*[11]. Cartilage fragments or extracts inhibit TAF-induced neovascularisation *in vivo*[3,4] and cartilage tumours are the least vascularised of all tumours tested[12]. The inhibitory effect of chondrocytes thus seems to be similar regardless of the type of stimulus used to induce neovascularisation. The stimulatory factors may be produced by lymphocytes as lymphokines or their production may be lymphocyte dependent. In practice, tumour-induced and lymphocyte-induced angiogenesis are indistinguishable[13]. TAF has been isolated from tumour cells[2] but in many tumours the extent of lymphocytic infiltration is rather high[14]. Tumours do not grow well in the

Table 1 The number of blood vessels evoked in (CFW×C57B1/Sn)F_1 mice by intradermal injection of parental or syngeneic lymphoid cells mixed with non-lymphoid parental or syngeneic cells

Cells injected	No. of vessels (mean + s.d.)	No. of injections	Conclusion
Parental spleen cells			
4×10^6	27.75±5.34	52	Angiogenesis all results differ
2×10^6	20.66±4.19	12	
1×10^6	15.83±3.24	12	
4×10^6 parental spleen cells mixed with parental chrondrocytes in ratio			
20:1	24.76±7.03	20	Weak inhibition of angiogenesis
10:1	18.96±6.08	30	Marked inhibition of angiogenesis
5:1	29.16±4.70	6	No inhibition
2:1	35.75±6.19	4	Stimulation
4×10^6 parental spleen cells mixed with parental kidney cells obtained enzymatically in ratio			
10:1	26.58±7.12	12	No inhibition
5:1	28.66±5.24	6	
4×10^6 parental spleen cells mixed with killed parental chondrocytes in ratio			
10:1	25.86±7.62	6	No significant inhibition
Parental thymocytes 4×10^6	12.15±7.62	13	Not different from 1×10^6 spleen cells
4×10^6 syngeneic (F_1) spleen cells	6.21±2.65	47	Control
4×10^6 syngeneic spleen cells mixed with parental chondrocytes in ratio			
10:1	5.35±3.81	6	Not different from control
or with syngeneic chondrocytes in ratio			
5:1	14.16±2.63	6	Weak angiogenesis
Syngeneic chondrocytes 8×10^5	4.00±1.41	4	Control

Significance at $P < 0.05$ level estimated by Student's t test.

anterior chamber of the eye or in perfused organs[15] where the access of lymphocytes to tumour cells is prevented. A rare spontaneous tumour growth in nude mice[16] could support this assumption, favouring Prehn's immunostimulation theory[17].

We found that at higher chondrocyte–lymphocyte ratios (1 : 5 or 1 : 2) an increase of angiogenesis rather than inhibition was seen, suggesting that an immunological reaction may be occurring against tissue-specific antigens on the chondrocyte surface. It has been shown that syngeneic rat chondrocytes can evoke an immune response suggesting that these chondrocytes possess a sequestered antigen[18–20]. The same is probably also true for murine chondrocytes.

Therefore, when chondrocytes and lymphocytes are mixed, two different processes may occur. At low ratios primarily an angiogenesis-inhibiting process occurs, but at higher ratios this is overcome by the increased production of lymphocyte-angiogenetic substances directed against chondrocyte-specific antigens which increases new blood vessel formation, rather than decreases it as would be expected.

The finding that viable chondrocytes can inhibit new blood vessel formation has some morphogenetic implications for osteogenesis. Capillaries from perichondrium cannot penetrate cartilage anlages of long bones. When a number of chondrocytes die in the ossification centre, the local concentration of the inhibitory substance decreases, which might permit localised invasion of capillaries of the periosteal bud and thus initiate osteogenesis.

In addition, we have tested the effect of 1 M HCl–guanidine extract of human costal rib cartilage (obtained from biopsy) on mouse endothelial cell growth induced by local injection of allogeneic murine lymphocytes. This extract also inhibited endothelial cell growth (unpublished) showing that the cartilage inhibitory effect can act across the xenogeneic barrier.

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Human smooth muscle autoantibodies reacting with intermediate (100 Å) filaments

THE specificity of human smooth muscle autoantibodies (SMA) is known only in chronic active hepatitis where SMA are of anti-actin type[1,2]. In other diseases there seem to be SMA of additional specificities[3–5]. Anti-actin antibodies react with microfilaments of cultured cells[6,7]. We describe here two human SMA-positive sera which react in indirect immunofluorescence microscopy (IFL) with other cytoskeletal structures, intermediate (100 Å) filaments. Such filaments are especially abundant in smooth muscle cells[8,9] but occur also in other cells including fibroblasts[10].

The SMA-positive sera were obtained from two cancer patients and reacted typically with smooth muscle tissue by indirect IFL technique[11] but could not be neutralised with purified actin. These sera reacted also with intercalated disks and the periphery of rat cardiac myofibrils. A similar

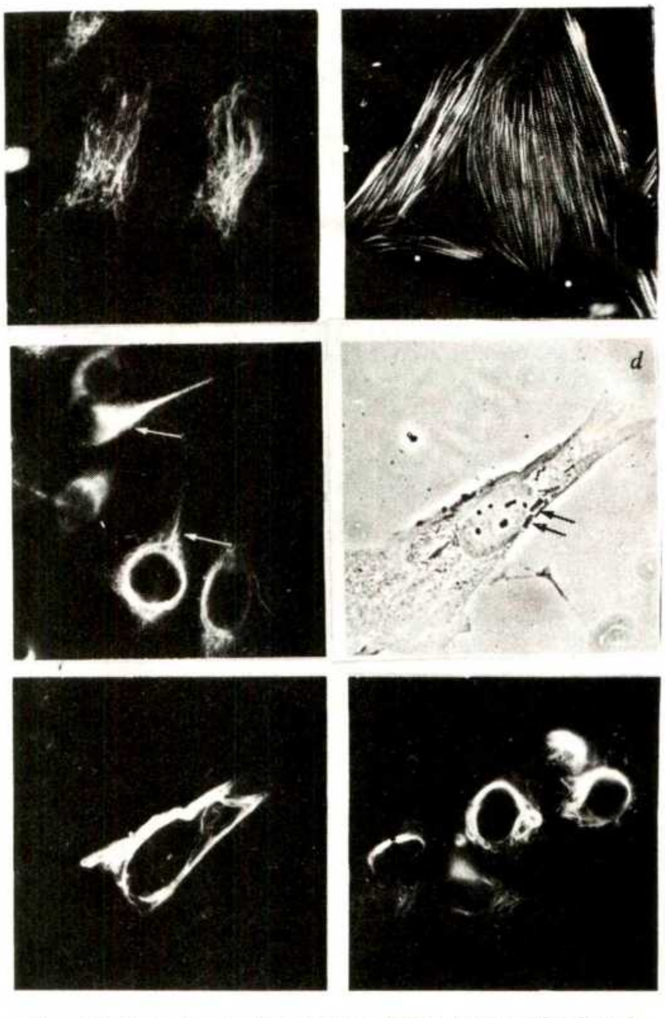

Fig. 1 Indirect immunofluorescence (IFL) staining of cultured cells with two human sera, one containing smooth muscle antibodies (SMA) to intermediate filaments (*a* and *c–f*) and the other to actin (*b*). Both sera had a SMA titre of 5,000 determined by indirect IFL using unfixed cryostat sections of rat stomach and kidney as substrates. To visualise human antibodies FITC-conjugated sheep anti-human immunoglobulin (NBL, Solna, Sweden, F/P molar ratio 2.9) was used. Human embryonic skin fibroblasts cultivated in RPMI 1640 + 10% foetal calf serum were used in their 10th to 25th passage *in vitro*. Mouse neuroblastoma cells were cultivated in MEM + 10% foetal calf serum. Cells were grown on cover slips for 48 h before fixation for 10 min in −20 °C acetone. *a*, Reaction of SMA of anti-intermediate filament type with fibroblasts. Note the fine fibrillar network in the cytoplasm. *b*, Reaction of SMA of anti-actin type with fibroblasts. The typical filamentous staining is seen. *c*, Reaction of SMA of anti-intermediate filament type with mouse neuroblastoma cells. Note the perinuclear bundles (arrows). *d*, A fibroblast, treated with vinblastine sulphate for 4 h (Lilly, 10 μg ml^{-1}), visualised in phase contrast microscopy. Tubulin containing paracrystals, induced by the treatment are indicated by arrows. *e*, The same cell as in (*d*) stained by SMA of anti-intermediate filament type. Coiling perinuclear bundles of fluorescence are seen. The paracrystals are not visualised. *f*, Reaction of SMA of anti-intermediate filament type with fibroblasts treated with demecolcine for 12 h (Colcemid, Ciba, 0.1 μg ml^{-1}). Thick coiling fluorescent bundles are seen at the perinuclear area also after this treatment.

staining pattern was recently reported by Lazarides and Hubbard[12] who used an experimental antiserum to 'desmin', a subunit protein of intermediate (100 Å) filaments.

In human embryonic skin fibroblasts our SMA-sera stained a fine fibrillar cytoplasmic network by IFL (Fig. 1*a*). This fluorescence pattern clearly differed from the microfilament staining produced by SMA of anti-actin type (Fig. 1*b*) and could not be inhibited with purified actin. A preparation of intermediate (100 Å) filaments isolated using the method of Cooke[8] completely inhibited both the fibrillar staining of fibroblasts and the staining of smooth muscle and cardiac tissues. To identify the fibrillar structures found in cultured fibroblasts by IFI we treated cells with cytochalasin B, vinblastine and demecolcine (Colcemid). These drugs induce typical alterations in the cellular cytoskeleton[10,13]. Cytochalasin B characteristically abolished the filamentous staining obtained with anti-actin antibodies but the fibrillar staining was not affected. Treatment with vinblastine resulted in the formation of paracrystals which are known to contain tubulin[14]. These remained unstained by IFL (Fig. 1 *d*, *e*). Instead, in cells treated either with vinblastine or demecolcine perinuclear coiling bundles were seen (Fig. 1*e*, *f*). Such bundles, induced by microtubule-disrupting drugs, consist of intermediate filaments[10]. This was confirmed in our study by electron microscopy of demecolcine- (Fig. 2) and vinblastine-treated cells.

When cultured mouse neuroblastoma cells[15] were stained by IFL, bright perinuclear bundles were seen (Fig. 1*c*). The bundles were similar to those observed by Jorgensen *et al.*[16] who used antibodies specific for a subunit protein of neurofilaments. The reaction of our sera with neuroblastoma cells corroborates the recent suggestions that neurofilaments and intermediate filaments are identical[16,17].

Our results show that treatment of cells with drugs characteristically altering intracellular organisation offers a method in studies of autoantibodies reacting with cytoskeletal structures. Using this approach we have found that some SMA react with autoantigens located in intermediate filaments. Thus far only one component of the intermediate filaments has been described, desmin[12] or skeletin[9], having a molecular weight of between 54,000 and 55,000. Whether our sera contain antibodies against this protein is not yet known.

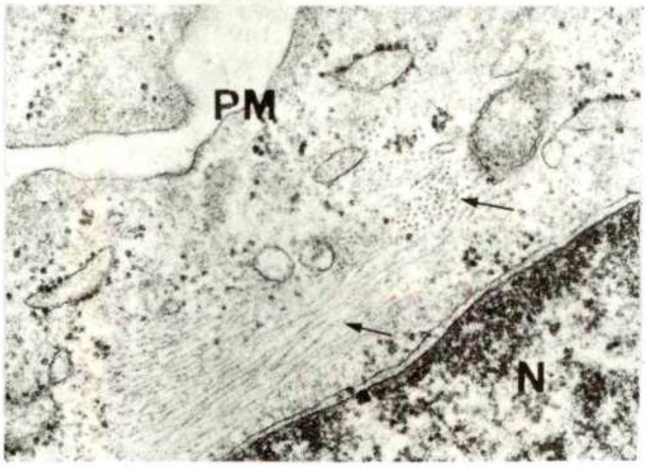

Fig. 2 An electron micrograph of a cell treated with demecolcine ($0.1\ \mu g\ ml^{-1}$) for 12 h. For electron microscopy the cultures were fixed in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate (*p*H 7.2), postfixed in 1% osmium tetroxide and embedded in Epon 812. Thin sections were transversally sectioned from cultures and post-stained with uranyl acetate and lead citrate. Electron microscopy was done at the Department of Electron Microscopy, Helsinki University. Bundles of filaments, 10 nm in diameter, are seen perinuclearly both tangentially and transversally sectioned (arrows). N, Nucleus; PM, plasma membrane. × 39,000.

Intermediate filaments have been studied mainly by electron microscopy[10,18,19]. Human autoantibodies offer an additional tool to study the function and suggested identity of these filaments in different cells.

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Myogenic defect in human muscular dystrophy

THE aetiology of muscular dystrophy has been considered to be either myogenic or neurogenic[1]. Much of the evidence has been obtained from studies of muscle in tissue culture using animal[2] or human[3] sources. Although results have often been equivocal[4,5] the most recent studies have failed to show any morphological differences between normal and abnormal muscle[3,6,7]. Hence, it could be argued that the primary defect is not in the muscle but rather may be in the nerve which innervates the muscle[6]. But, almost all of these studies have involved techniques based on explants of small muscle pieces, which muscle fragments then typically require one week for outgrowth from the tissue pieces and a further week or two before differentiated function (myotube formation) is seen[3,6]. For expression of maximally differentiated cultures (cross striations and spontaneous contractions) it has been presumed that innervation may be required, since contractions are rarely found and are essentially seen only in the presence of added nerve cells[6]. We have recently described a new technique for establishing fully differentiated adult muscle cultures from mononucleated cells and have found spontaneous contractions without the requirement for nerve cells[8]. Using this method, we have compared the maturation of muscle from the Duchenne (X-linked recessive) form of muscular dystrophy with appropriate control cases, and have found an abnormality in cellular behaviour restricted to the Duchenne material.

Table 1 shows the results from cultures of nine dystrophic patients and nine controls and demonstrates that in each case the abnormal formation of clustered cells occurs before the appearance of myotubes. It also demonstrates that the level of creatine phosphokinase (CPK) specific activity is generally lower than in controls. The first sign of abnormality can be detected as early as four days after plating the cells and consists of areas where the cells are clustered together in close proximity (Fig. 1*a*) rather than being more uniformly distributed in a monolayer (Fig. 1*b*). Some of the cells in the clusters had a superficial resemblance to fibroblasts, but more careful examination revealed that they were quite atypical in their morphology. The nuclei of these cells as well as the perinuclear cytoplasm seemed to be increased in size and hence might be termed hypertrophic, as previously described[9,10]. They also have longer processes (Fig. 1*a*). There is, however, a proportion of myoblasts which fuse in apparently normal fashion.

With further differentiation there are a certain number of refractile myotubes but their distribution is again abnormal in that they typically span the gap between two of the multilayered cell clusters which are by now several cells thick (Fig. 1*c*) and thus give the appearance of a pyramidal form of multicellular complex when one focuses up and down along their margins. Of course, the photographs do not directly reveal this third dimensional consideration. The clear areas on the surface of the culture plate between the hypertrophic clusters are still prominent at this stage, which is again quite different from the normal muscle (Fig. 1*d*) or from the other diseases referred to previously. Within these cases there was no obvious correlation between the degree of connective tissue found in the biopsy and the degree of cluster formation; quite the contrary—the patient (M.C.) with a massive excess of fibroblast infiltration took the longest time (Table 1) to exhibit the abnormality.

Although there have been various comments on the behaviour of dystrophic muscle in culture, other authors have not described these particularly early abnormal events during the maturation sequence. It is thus quite different from the normal sequence whereby single myogenic cells fuse into multinuclear myotubes and then differentiate into fully refractile muscle straps[11]. Those techniques which initiate cultures from small explants of tissue pieces have a requirement for cell migration from the initial explants, before the myoblasts can begin to fuse and form myotubes. This difference in the methods for starting the cultures may explain in part why explant techniques obscured these early events. But, the technique which starts with single cells does not select for cells which must migrate out of the explant before they can differentiate into myotubes. A closer approximation to starting cultures with single cells has been the technique which begins with smaller 'muscle cell pieces' ($60 \times 25\ \mu m$) each of which contains a few nuclei that grew initially by 'budding'[12]. This technique revealed some abnormal intracytoplasmic inclusions but the abnormalities in cellular distribution and myotube formations were not described[13].

Concerning the primary metabolic lesion, there are several possible defects in cellular metabolism which give rise to the abnormal muscle development (for example, membrane alterations[7,14,15]). But it should also be considered that the genetic defect may involve an abnormality of control gene which normally initiates a specific sequence (for example, during the orientation of thick and thin filaments) during cellular differentiation[16].

It should not yet be concluded that the abnormalities are diagnostic for Duchenne dystrophy. The origin of the cells may be an abnormal form of fibroblastic connective tissue. They may also be aberrant precursors of myoblasts, however.

The advantages of the current technique are that one has ilve cells which can be collected for specific metabolic assays, that certain cells display an unusual form of cell–cell interactions, that the cells within the unusual clusters do not seem to resemble normal fibroblastic or epitheliod morphologies[17] and that the complete sequence of muscle maturation[11] can be observed and sampled at appropriate developmental times. Further, since these abnormalities

Table 1 Comparative data on dystrophic muscle biopsies and controls run in the same culture conditions

Patient	Age (yr)	Diagnosis	No. of Petri plates	Clusters at DIV	Myotubes at DIV	Cells collected DIV	Specific activity of CPK
J.W.*	5	Duchenne	5	7	8	15	0.2
G.E.	4	Duchenne	3	4	8	14	0.5
J.B.†	4	Duchenne	2	5	8	12	0.76
K.H.	3	Duchenne	8	4	CON	5	CON
M.C.‡	7	Duchenne	2	10	11	15	0.30
S.F.	4	Duchenne	9	6	8	13	0.50
S.W.	4	Duchenne	5	8	11	13	0.75
P.M.	23	Becker	2	9	9	14	0.1
M.P.	7	Becker	2	7	10	14	0.9
Controls							
A.D.*	15	spinal muscular atrophy (denervation)	2	NS	11	15	1.0
A.T.†	2	spinal muscular atrophy (denervation)	7	NS	8	12	0.73
E.H.‡	3	hereditary ataxia, normal muscle	2	NS	10	15	1.2
M.M.	2	normal muscle	2	NS	21	29	2.3
J.D.	4	nonspecific myopathy	4	NS	9	14	0.6
D.C.	7	fascioscapulohumeral dystrophy	5	NS	8	15	0.4
G.S.	6	nonspecific myopathy	4	NS	10	16	1.5
D.T.	2	nonspecific myopathy	4	NS	10	15	1.5
J.M.	7	Kugelberg–Welander	1	NS	15	22	0.01

CON, Contamination; DIV, days *in vitro*; CPK, creatine phosphokinase activity in μmol creatine per min per mg protein at 25 °C; NS, not seen.

*, †, ‡, Cultures run in identical conditions, that is, biopsy received the same day, same digestion, same media changes and so on.

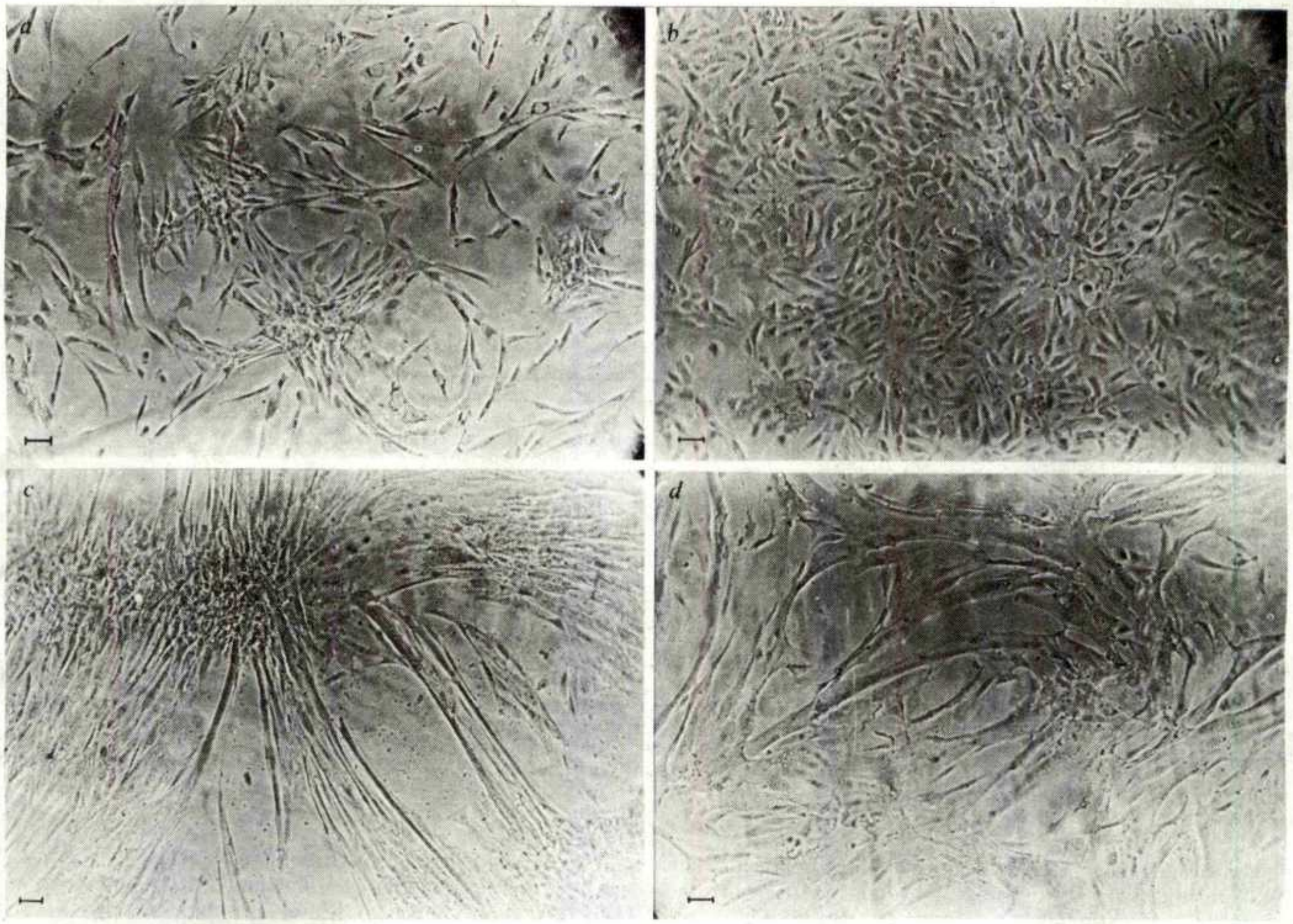

Fig. 1 General culture methods were as before[8]. Muscle was weighed and then submitted to four serial incubations with a mixture of 0.1% collagenase and 0.2% trypsin in a phosphate-buffered saline at 37 °C. The cells (6×10^4 per dish) (over 95% viable by dye exclusion) were plated on collagen-coated 35-mm Lux dishes in Dulbecco's Modified Eagle's Medium containing 10% (v/v) screened horse serum and 2% (v/v) detoxified chick embryo extract. *a*, Typical low power view of the hypertrophic clusters (see text for full description) from a Duchenne form of muscular dystrophy (JB). Culture was 6 d *in vitro*. *b*, Control culture for (*a*). Case of spinal muscular atrophy of similar age grown in identical culture conditions (A.T.); 6 d *in vitro*. *c*, Same case as (*a*) (Duchenne muscular dystrophy) but 11 d *in vitro* now showing abnormal muscle straps which span between hypertrophic clusters (see text for full description). *d*, Same case as (*b*) (control but 11 d *in vitro*. Scale bars 50 µm.

are found in the absence of any nerve cell input, at least this portion of the genetic defect is myogenic rather than neurogenic.

Clearly, further work is required to specify the precise biochemical alterations associated with these abnormalities in the initial cell appearance, their subsequent intercellular behaviour (and possibly cell membranes[7]) as well as their final degree of differentiation, including response to co-cultured nerve cells.

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A human leukaemic cell expressing hybrid membrane phenotypes

INDIVIDUAL surface markers differentiate B and T cells with varying degrees of certainty, and it is generally agreed that a panel of immunological markers is required to assign a particular cell to a category[1]. In general, the ability to form spontaneous sheep red blood cell rosettes (E) and to react with anti-T-cell antisera are characteristics of T lymphocytes, whereas B lymphocytes are characterised by the presence of surface immunoglobulin (SmIg), C3 and Fc receptors and the presence of Ia-like antigens on the cell membrane[2,3]. However, in some instances lymphocytes are detected which exhibit combined B- and T-cell markers[4]. This has been particularly true in the case of human leukaemic cells, where a variety of unusual combinations of phenotypic markers has been described[5,6]. In this communication, we report on a human leukaemic cell from a case of hairy cell leukaemia[7–9] (HCL) with unusual combined B- and T-cell membrane characteristics.

In Table 1, data are presented on the surface marker characteristics of the unusual hairy cells (HCs) from peripheral blood and spleen when tested over a period of two months. Although the expression of these individual B and T phenotypic markers fluctuated over this time, a significant overlap was found in the values obtained when the T cell markers (E-rosetting and anti-T cell serum) and the more conventional B cell markers (SmIg, Fc(γ) and the Ia-like protein complex P29/34) were compared. The summation of these B- and T-cell markers greatly exceeded 100%. This contrasts with the only reported case of a biclonal T- and B-cell leukaemia, in which the two clones independently expressed B and T membrane phenotypes[10]. In addition to the data presented in Table 1, the cells were found to be negative for the C3b receptor[4] and the EB virus nuclear antigen (EBNA)[11], but did form spontaneous rosettes with mouse erythrocytes, a marker for a subpopulation of B cells[12] (1–9% with peripheral blood cells and 47% with splenic cells). In the spleen cell preparation, which was shown to contain >95% HCs, 95% of the cells were stained for intracytoplasmic IgM, IgG and *k* chain, of which 25% formed an intense patchy staining with FITC-labelled anti-IgG and anti-IgM. The finding of a receptor for IgM (Table 1) on these cells is not surprising, since this receptor is present both on a subpopulation of normal T lymphocytes[13] and on the pathological cells of some B-cell leukaemias[14] including HCL (ref. 9). The distinctive hairy appearance of the pathological cells of HCL enabled easy recognition of these cells both at light and electron microscope level in the various marker studies.

Table 1 Cell surface marker studies* on isolated mononuclear cells from peripheral blood and spleen

Source of mononuclear cells	Date	E	Anti-T	Percentage of cells rosetting or staining SmIg μ	δ	γ	α	*k*	λ	Fc (μ)	Fc (γ)	P29/34
Peripheral blood	26.1.77	76	—†	5	18	5	—	19	3	22	—	—
	12.2.77	79	75	11	44	10	—	52	4	—	65	—
	26.2.77	52	70	77	37	72	0	70	16	66	78	79
	12.3.77	84	—	—	33	—	—	34	—	—	47	—
Spleen	12.1.77	73	—	5	82	—	0	78	0	—	96	90

*Peripheral blood (PB) mononuclear cells were separated by Ficoll–Hypaque centrifugation; a splenic cell suspension was prepared by forcing pieces of fresh spleen through a wire mesh and the mononuclear cells isolated using a Ficoll–Hypaque mixture. E-rosettes were performed by the method of Kaplan and Clarke[19]; the specific rabbit anti-T serum[20] (a gift from Dr G. Janossy) was employed using an indirect fluorescent method; SmIg was detected either by the rosette method of Ling *et al.*[21] (purified sheep antisera a gift from Dr N. Ling) or by direct immunofluorescence (rabbit antisera a gift from Dr J. Smith); the Fc(μ) receptor was detected using rabbit IgM by the rosette method of Burns *et al.*[9]; the Fc(γ) receptor was detected using rabbit IgG by a rosette method; rabbit antiserum to purified human P29/34 (ref. 21) (a gift from Dr T. Springer) was used in an indirect fluorescent method (positive controls performed with B-cell leukaemias and negative controls with a T-cell line and a case of T-cell Sezary syndrome).

†Not done.

In the experiments to detect SmIg, a pre-incubation period of 1–2 h at 37 °C was routinely adopted, and the cells were observed to express SmIg of a single light chain type (*k*), of which IgD (*k*) was shown to persist after seven days in culture. The SmIg could also be removed by capping with anti-*k* chain serum. These points make it unlikely that SmIg can be attributed to cytophilic Ig or to autoantibody against the leukaemic cell.

To establish the nature of the E-rosette, a variety of blocking and inhibition experiments were carried out (Table 2). Whereas E-rosette formation was blocked by the anti-T-cell serum (although this antiserum did not interfere with the detection of SmIg) it was not blocked by the anti-P29/34 serum. Furthermore, a reduction in E-rosette formation by HCs, similar to that seen with normal T lymphocyte controls, was observed when rosette formation was carried out at 4 °C, with and without centrifugation, and in the presence of sodium azide or cytochalasin B. Thus, like E-rosette formation by normal T lymphocytes[15], this E-rosette formation was energy dependent and required an intact microfilament system. In addition, E-rosette formation was not markedly blocked either by group A substance from human saliva (which would block antibody activity against a heterophile antigen) or by treatment with anti-Ig sera. In the latter case, a SmIg receptor for sheep erythrocytes was further excluded by removing SmIg in a 'capping' process. In conditions in which no *k* chain was detected on the cell, E-rosette formation still occurred and, in some instances, the number of leukaemic cells rosetting with erythrocytes rose significantly.

HCL, a chronic leukaemia related to chronic lymphocytic leukaemia, is now considered to be a B-cell neoplasm[7,8] but the cells have been reported as possessing a

Table 2 The effect on E-rosette formation of blocking with various substances and of capping of SmIg

Type of experiment	Percentage sheep RBC (E) rosette formation* Control	Anti-μ†	Anti-δ†	Anti-γ†	Anti-*k*†	Anti-T‡	Anti-P29/34	Sodium azide (0.1M)	Cytochalasin B (20 μg ml^{-1})
Blocking	52	53	36	52	59	0	59	—	—
	79	—	77	—	72	4	—	18	54
Capping§	52	69	66	82	65	—	—	—	—
	77	77	65	—	66				

*Patient's leucocytes did not form spontaneous rosettes with a variety of other heterologous RBCs including ox, rabbit, guinea pig and rat.

†Anti-SmIg sera used at a concentration which totally inhibited SmIg–anti-SmIg rosette formation[21].

‡This anti-T-cell serum[20] did not affect SmIg–anti-SmIg rosette formation.

§SmIg shown to have capped with each of the antisera by direct immunofluorescence and by failure to form SmIg–anti-SmIg rosettes.

number of monocytoid features such as adherence to glass and phagocytic ability[7–9]. Furthermore, the pathological cells of most cases of HCL are of indeterminate maturity since they show such features of immaturity as poorly condensed peripheral nuclear chromatin[9] and the absence of a receptor for cholera toxin[16]. The nature of the disease, however, and the capacity to produce Ig for secretion suggests involvement of a mature cell type. It may not be surprising, therefore, that a cell of mixed B and T phenotype should appear in a leukaemia which is unusual in so many other ways, and it may indicate that the neoplastic transformation occurs at a point close to the divergence of B and T cells from the common haemopoietic stem cell. Alternatively, it may be that the genes controlling the expression of receptors for T-cell antigen have become derepressed by the neoplastic process acting on the HCs although derepression by EB virus is less likely due to the absence of EB nuclear antigen in these cells.

The argument of gene derepression has been proposed as the mechanism in the only previously described case of a human leukaemic cell, from a case of lymphosarcoma cell leukaemia, which possessed both SmIg and T-cell features[17]. Although there are some reports of leukaemic cells both forming E-rosettes and possessing receptors for Fc or C3, (refs 5, 6), there is evidence that subpopulations of normal lymphocytes also possess these marker combinations[4] and such leukaemias may be an amplification of these minor subpopulations. No normal lymphocyte population bearing the marker characteristics of the leukaemic cells in this report has yet been described in man, but such cells may exist in the mouse[18].

In conclusion, a case of HCL is described in which the pathological cells were shown to be E$^+$, anti-T$^+$, Fc(μ)$^+$, SmIg$^+$, cytoplasmic Ig$^+$, P29/34$^+$, Fc(γ)$^+$, mouse erythrocyte rosette$^+$, C3b$^-$ and EBNA$^-$. This observation emphasises the importance of using a large panel of immunological markers when studying membrane marker characteristics on haemic cells from both the normal and leukaemic situation. Indeed, cases of this kind could reveal further information on the nature of the leukaemic transformation and the study of such leukaemic cells may well lead to the identification of subpopulations of normal haemic cells.

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Epidermal Langerhans cells bear Fc and C3 receptors

THE mammalian epidermis contains a system of dendritic cells, eponymously referred to as Langerhans cells, whose functions are obscure. It is, however, accepted that Langerhans cells are of mesenchymal origin and since they are known also to occur in the dermis, lymph nodes, thymus, and other organs, the concept of Langerhans cells has widened to encompass a cellular system populating the connective tissues and certain stratified squamous epithelia of mammals[1]. Among the many hypotheses about their biological significance they have been considered to represent immunocompetent cells or cells which might capture antigenic materials[2], but there is insufficient evidence for this. Lymphocytes have been observed to be closely associated with Langerhans cells in delayed type hypersensitivity reactions to dinitrochloro-benzene in guinea pigs[3]; and when guinea pigs passively sensitised with lymphocytes from ferritin-hypersensitive donors, were challenged with ferritin, ferritin-containing Langerhans cells were observed in the draining lymph nodes of the challenged animals[4]. Metals and certain amines have an affinity for Langerhans cells *in vitro*[5], but since they can phagocytose ferritin and other substances without previous sensitisation[1], doubt remains whether they are immunologically engaged in the uptake and transport of potentially antigenic material. If Langerhans cells are indeed involved in immunological reactions, they should bear some of the surface markers characteristics of cells with known immunological functions, and we have now shown that they carry receptors for Fc and C3.

Epidermis of human volunteers was separated from the dermis, *in vivo*, using a suction blister device[6], washed in Medium 199 (Flow Laboratories, Irvine, UK), and dissociated, *in vitro*, with 0.5% trypsin (Flow Laboratories, Irvine, UK) in phosphate-buffered saline (*p*H 7.2) for 30 min at 37° C. Epidermal cell suspensions were prepared by teasing and pipetting the epidermal specimens gently in Medium 199 supplemented with 50 mg ml^{-1} gentamycin and 20% foetal bovine serum. After a double rinse and resuspension, the viability of the epidermal cells, checked by Trypan blue exclusion, ranged from 92 to 96%. The epidermal cells were then incubated with indicator reagents for Fc and C3 receptors, according to rosette assays described previously[7]: equal amounts of the epidermal cell suspension ($5–10 \times 10^6$ cells per ml) and of a 1% suspension of either IgG coated bovine red blood cells (EA-IgG) or IgM and complement-coated bovine red blood cells (EAC) were mixed, centrifuged and then incubated at 0 °C for 1 h. Any cell binding three or more erythrocytes was scored as positive, a minimum of 200 cells being counted.

Multiple experiments consistently showed that 3–4% of the cells in the epidermal cell suspensions formed rosettes with the EA-IgG and the EAC reagent. Rosetting was not seen in the controls, which included incubation of epidermal cells with untreated bovine red blood cells (BRBC); BRBC coated with IgM antibody (EA-IgM); EA-IgM incubated with heat-inactivated or EDTA-inactivated fresh mouse serum; in the case of EA-IgG, epidermal cells pre-incubated with 10 mg ml^{-1} heat aggregated human IgG or reacted with BRBC, coated with Fab$_2$ fragments of anti-BRBC IgG antibody. For this purpose the IgG anti-BRBC antibodies were pepsin digested according to Nisonoff *et al.*[8] and passed over a Protein A column (Protein A Sepharose CL 4B, Pharmacia, Uppsala, Sweden) to remove possible residual intact IgG molecules. BRBC coated with this Fab$_2$ antibody preparation (adjusted to the protein concentration of the original total IgG preparation) displayed no reactivity with either mononuclear blood cells or cells from epidermal cell suspensions.

Since the demonstrations of EAC rosettes after trypsin treatment contrasts with reports that EAC receptors on leukocytes are trypsin sensitive[9], the influence of our trypsinisation procedure was checked on peripheral mononuclear cells. Whereas a 0.005% concentration of trypsin destroyed the C3 receptors on lymphocytes, EAC rosette formation with latex-ingesting monocytes was still detectable after treatment with 0.5% trypsin. Thus, the trypsin sensitivity of EAC rosette-forming cells from epidermal cell suspensions corresponded to that of latex-ingesting monocytes from the peripheral blood.

To determine the nature of the subpopulation of epidermal cells showing EA-IgG and EAC rosette formation, smears were prepared from cell suspensions treated with EA-IgG and EAC and incubated according to a modified[1] Wachstein and Meisel cytochemical technique[10] for the demonstration of nucleosidetriphosphatase (NTP). This enzyme is, in the conditions used, a cytochemical marker for Langerhans cells[1]. All rosette-forming cells of the epidermal cell suspensions were reactive for NTP, whereas the non-rosetting cells failed to show enzymatic activity. In addition, epidermal cells pelleted after exposure to the EA-IgG reagent were processed for electron-microscopy, either directly or after pre-incubation for NTP cytochemistry. At the ultrastructural level, all rosette-forming cells exhibited NTP activity, whereas non-rosetting cells did not; rosetting cells exhibited the classical ultrastructural morphology of LC, including the typical LC cell granules (Fig. 1), whereas non-rosetting cells showed morphological features of either keratinocytes (keratinising epidermal cells) or melanocytes. These results show that the subpopulation of epidermal cells which have the receptor for Fc and C3 are Langerhans cells.

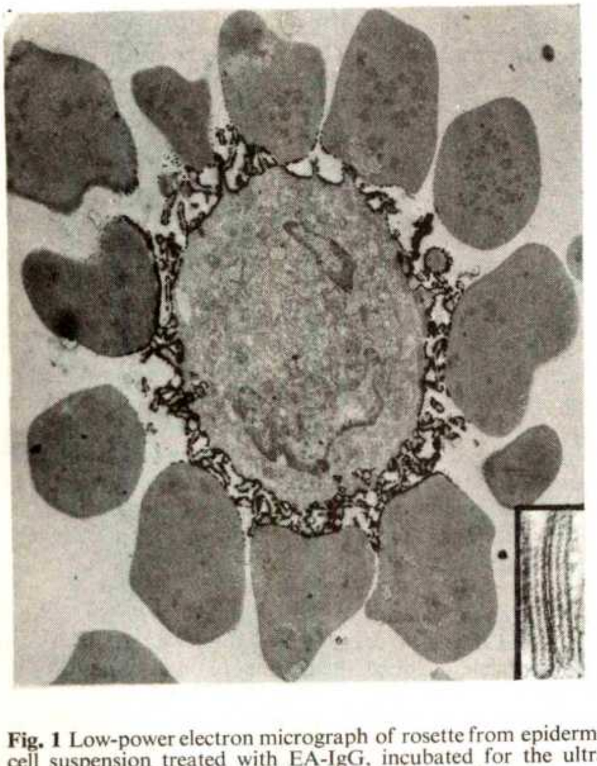

Fig. 1 Low-power electron micrograph of rosette from epidermal cell suspension treated with EA-IgG, incubated for the ultrastructural demonstration of NTP, and processed for electron microscopy. The rosetting, Fc receptor-bearing cell exhibits a folded nucleus, dendritic and villous surface projections, reaction product indicating NTP activity on its membrane and typical Langerhans cell granules in its cytoplasm (inset).

Fc and C3 receptors are known to occur on lymphocytes[11], on monocytes–macrophages histiocytes[12], and, as recently shown, on isolated hepatocytes[13]. Since Langerhans cells bear neither morphological nor cytochemical characteristics of lymphocytes[1] and since preliminary studies in our laboratories indicate that they exhibit neither membrane immunoglobulins nor E receptors, they do not qualify as lymphocytes. On the other hand, their Fc and C3 receptors make them reasonably good candidates for inclusion in the monocyte–macrophage–histiocyte series of cells despite the fact that, in phagocytosis experiments, *in vivo*, they fail to exhibit greater phagocytic capacity than the other cells of the epidermis[14]; also, the morphological identity of Langerhans cells with certain histiocytic cells in histiocytosis X (refs 1, 15) a proliferative histiocytic disease in man, is a good morphological correlate to these immunological findings. Our findings thus provide evidence for previous suggestions[2,4,5] that Langerhans cells perform macrophage-like functions.

The cells of the monocyte–macrophage histiocyte system are known to have an important role in immune reactions. They are responsible for the uptake, processing, and transfer of antigens to immunocompetent lymphocytes[16,17], and they are involved in T–B interactions[18]. The biological role of their Fc and C3 receptors is not entirely clarified, but they seem to mediate antibody-dependent cellular cytotoxicity[19] and the phagocytosis of immune complexes, whereby the C3 receptors bind C3 sites of immune complexes, and the Fc receptors are responsible for the ingestion phase[20]. The antibody-binding capacity of macrophages may thus regulate the inductive phase of the immune response since cell-bound antibody can mediate the presentation of antigen to T lymphocytes[21].

It does not appear unreasonable to assume that LC by means of their immunological receptor sites, perform functions similar to those of the monocyte-macrophage-histiocyte system, both by initiating the immune response to external antigens and by facilitating an enhanced reaction in the secondary response. These functions could thus provide a reasonable explanation for the paradox that Langerhans cells, as mesenchymal cells, are located in the most peripheral, ectodermal tissue of the mammalian organism, in the epidermis. If our conclusions are correct Langerhans cells may represent the most peripheral outpost of the afferent limb of the immune system.

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Ia antigen expression on human epidermal Langerhans cells

It has been proposed that the mammalian epidermal Langerhans cell is an epidermal equivalent of the cells of the reticulo-endothelial system, which are involved in binding and presentation of allergens in lymph nodes and the spleen[1,2]. The demonstration that these resident cells are capable of binding various allergens and migrate to the lymph vessels and local draining nodes, indicates a major function in the afferent arm of the primary immune response[3,4] and has improved understanding of contact allergic hypersensitivity reactions. Much still remains to be discovered concerning the characteristics of the cell type, its reactivity with various antigens, and its relationship to other cells of the immune system. We show here that epidermal Langerhans cells react positively by immunofluorescence with antisera raised in rabbits against human B lymphoblastoid cell line membrane glycoproteins, which indicates the presence of Ia-like antigens on these dendritic cells.

The Ia antigens are surface glycoproteins associated with particular groups of immunologically active cells[5-7]. Although they have been detected in small quantities on T cells they seem to be associated primarily with B cells[8,9]. Recent studies have, however, shown them to also be expressed on macrophages[10], sperm, foetal liver cells and epidermal cells[11]. This last group is intriguing, since the observation was made by means of cytotoxic assays. Thus, no morphological distinction was made between the various cell types present in the epidermis. The proposal that Langerhans cells are a kind of histiocyte and the evidence pointing to the presence of significant numbers uniformly distributed in the epidermis[12], suggested that they might express Ia antigen. Since the functional significance of Ia antigens is not yet clear, confirmation of this proposal might have important implications. Ia antigens have been shown to be involved in mixed lymphocyte reactions (MLR)[13,14], graft-versus-host (GVH) reactions[15], skin graft rejection[16] and a variety of other cooperative phenomena.

Human B-lymphocyte Ia antigens were isolated from B lymphoblastoid cell lines (Fig. 1). The final protein peak used for production of rabbit heteroantisera, had a molecular weight of 33,000 daltons. The heteroantiserum after absorption with a human T cell line (MOLT 4), was shown to react against B cells but not T cells or thymocytes. When incubated with lymphocytes in the MLR, the antiserum suppressed thymidine incorporation. In precipitin analysis in Ouchterlony plates, a rabbit antiserum to HL-B antigens (Dr H. Kunkel, Rockefeller University), showed immunological identity indicating a relationship to the Ia system.

Human skin obtained at surgery was frozen and 5-nm sections were prepared for direct and indirect immunofluorescence (IF) with the rabbit anti-Ia IgG fraction. Table 1 illustrates results of the various IF tests performed.

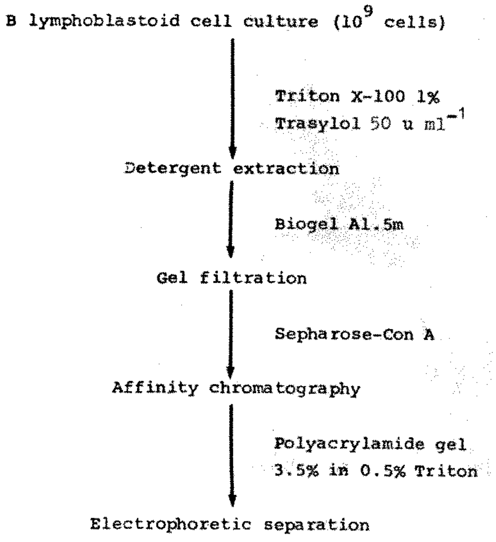


Fig. 1 Scheme of fractionation.

The findings can be summarised as follows: dendritic cells in the suprabasal zone of the human epidermis stain strongly at titres down to 1/1000 (Fig. 2). Specific immune blocking and pre-incubation with antigen confirmed the specificity of the reactions. The possibility that Fc receptors on Langerhans cells might nonspecifically bind immune reagents was tested using Fab′ fragments of the rabbit anti-Ia antiserum. Positive staining was maintained using this reagent. Experiments to demonstrate either Fc receptors or receptors for C'_3 using EA and EAC rosetting methods[18] on frozen sections were negative. Other workers have failed to show binding of EA rosettes to cells in the epidermis, that might be interpreted as Langerhans cells (R. Edelson, personal communication).

In order to study further surface receptors on Langerhans cells a cell suspension of EDTA separated epidermis was prepared by 30-min incubation at 37 °C in 0.5% buffered trypsin. Epidermal cell suspensions were checked for viability, for morphology with haemotoxylin and eosin, for ATPase staining and for the presence of Fc and C_3 receptors. EA (IgG) and EAC (IgM) rosetting methods were carried out on the cell suspensions with appropriate controls, that is, untreated SRBC and EA (IgM). With both EA and EAC methods between 2–3% of the cells formed rosettes of 5+ SRBCs. Rosetting was not seen in any of the controls. Thus cell suspensions of the epidermis prepared with trypsin demonstrate a small percentage capable of forming rosettes, indicating the presence of Fc and C'_3 receptors on their surface.

Table 1 Fluorescent antibody staining results

Substrate	Procedure	Reaction	Comment
A Normal human skin	1 R-anti Ia*→GAR-FITC	+	Positive suprabasal dendritic epidermal cells
	2 Preimmune R-serum →GAR-FITC	−	plus
	3 Fab′ of R-anti Ia→GAR-FITC	+	Histiocytes and some lymphocytes in dermis positive
	4 R-anti Ia→R-anti Ia FITC	−	
	5 R-anti Ia-FITC	+	
	6 GAR−FITC	−	
	7 Ia antigen+R-anti Ia→GAR-FITC	−	
B Depigmentary conditions for example, vitiligo, halo nevus	8 R-anti Ia→GAR-FITC	+	Main basal dendritic epidermal cells positive
C Rat keratinising epithelia skin, oesophagus	9 R-anti-Ia→GAR-FITC	−	No species cross-reactivity

***R-anti Ia, IgG fraction of rabbit anti-human Ia heteroantiserum.**
GAR-FITC: IgG fraction goat anti-rabbit IgG conjugated to fluorescein isothiocyanate.

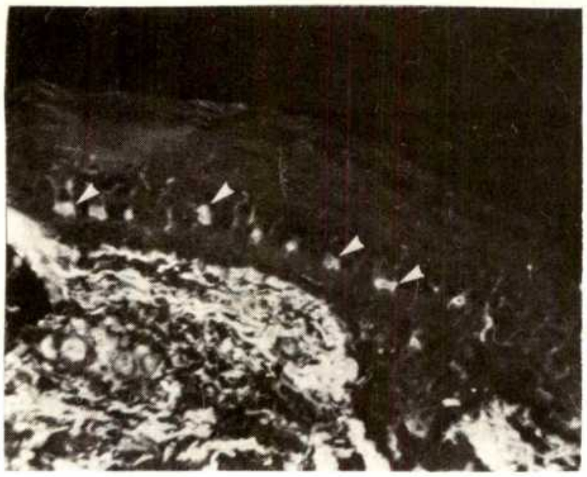


Fig. 2 Indirect immunofluorescence staining of normal human skin. Rabbit anti-human Ia (gG fraction) followed by goat anti-rabbit IgG–FITC positively stained suprabasal dendritic cells. ×238 (arrows).

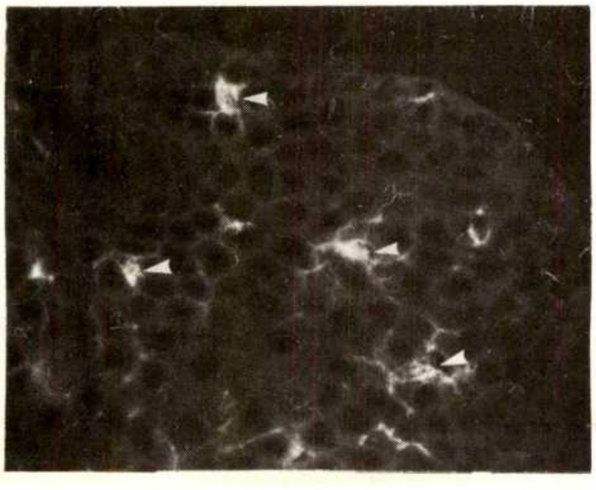
In addition, the same approximate percentage of cells show staining for ATPase and for Ia antigen as carried out by fluorescent microscopy. Simultaneous demonstration of the two reactions has not yet been achieved, but seems to be technically feasible.

As might be predicted from the information concerning distribution of Langerhans cells in leukodermatous[19,20] conditions such as vitiligo and halo nevi, staining reactions were noted not only in the suprabasal location but also in the basal layer. Furthermore there was no cross-species reactivity since Langerhans cells in rodent keratinising epithelia did not stain.

These observations suggest that if the Langerhans cell is a histiocyte, it is atypical, since it does not seem from frozen sections, to express either Fc or C'_3 receptors that are detectable by rosetting, nor is it very phagocytic[21]. Clearly the disparity in the detection of surface receptors on isolated cells as opposed to frozen sections can be explained either by the sensitivity of the methods or because of an unmasking effect of the trypsinisation. Whether the cells *in vivo* express these surface receptors requires further investigation. The specific staining with anti-Ia antisera should provide a tool to assist in the isolation of the Langerhans cell.

To confirm these findings ultrastructurally, we are using Fab′ fragments of the anti-Ia antiserum labelled with ferritin in electron microscopic studies. Preliminary results demonstrate a weak but specific staining of the membranes of epidermal Langerhans cells. In the same studies dermal histiocytes and some lymphocytes also show specific staining. Clearly the present identification of positively stained cells in the epidermis as being Langerhans cells relies heavily on the established suprabasal localisation in normal tissue and is supported by the changes in distribution seen in leukodermatous skin. The cells as shown in a tangential section are clearly dendritic (Fig. 3). ATPase-stained tissue sections show an identical distribution pattern.

Fig. 3 Indirect immunofluorescence staining of a tangential section of normal human skin (as in Fig. 2). High magnification demonstration of dendritic cells. ×680.



These observations provide interesting avenues for research on the Langerhans cell itself, as well as confirming that they express surface antigens that have so far been associated with immunologically active cells. They also have a role with such cells in the rejection of skin grafts.

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Epidermal Langerhans cells express Ia antigens

THE major histocompatibility complex (MHC) of mouse and man controls the expression of several cell surface antigens[1]. The classical transplantation antigens are present on most if not all adult, nucleated cells. However, molecules controlled by the immune response region of the MHC display a much more restricted tissue distribution. The immune response-associated antigens (the Ia antigens in the mouse and HLA-D antigens in man) seem to be integral parts of the plasma membrane of B and T lymphocytes, macrophages, spermatozoa, and epidermal cells[2]. The physiological role of the Ia antigens is far from understood but their participation in various immunobiological events is well documented (for a review, see ref. 3). In addition to their role as triggers in the mixed leukocyte reaction[4], the Ia antigens are required for collaboration between T and B lymphocytes[5] and between macrophages and T cells[6]. Since all known functions of the Ia antigens pertain to the immune system it seemed that they should be expressed on an epidermal cell population involved in immune reactions. We therefore set out to investigate which of the three cell types of epidermis, keratinocytes, melanocytes, and Langerhans cells, expresses the Ia antigens, and from the results of fluorescent antibody staining we conclude that it is the Langerhans cells.

Epidermal cells remove anti-Ia antigen antibodies from alloantisera[2] but no data are available as to the biochemical nature of the Ia antigen-reacting molecules in epidermis. To reveal the structure of these molecules biopsies of skin were obtained from patients undergoing surgery for mammary hyperplasia. The epidermis was separated mechanically from underlying dermis after heating the biopsies to 52 °C. The biopsies were treated by homogenisation on 0.02 M Tris-HCl buffer, *p*H 8.0, containing 0.15 M NaCl and 1% Triton X-100. Insoluble material was removed by centrifugation at 105,000*g* for 60 min and glycoproteins, present in the supernatant, were isolated on an affinity chromatography column containing covalently-bound Lens culinaris haemagglutinin[7]. Purified glycoproteins were labelled with ^{125}I and Ia antigen-reacting material was isolated by indirect immunoprecipitation using a specific rabbit anti-HLA-D antigen serum[8]. The immune complexes were dissolved and separated by SDS–polyacrylamide gel electrophoresis, as previously described[7]. Figure 1 shows that the anti-HLA-D antigen serum preferentially precipitated two types of polypeptide chains with the apparent molecular weights 28,000 and 34,000. This two-chain pattern is indistinguishable from that obtained when the same antiserum was used to precipitate molecules obtained from leukaemic B lymphocytes. However, two additional components with the apparent molecular weights 70,000 and 50,000, respectively, were also visualised. Since the same polypeptide chains were also present in precipitates generated by normal rabbit serum, it seems reasonable to conclude that they represent nonspecifically-precipitated contaminants. Thus, the result is consistent with the view that HLA-D antigens of epidermis have the same overall structure as their counterparts on B lymphocytes.

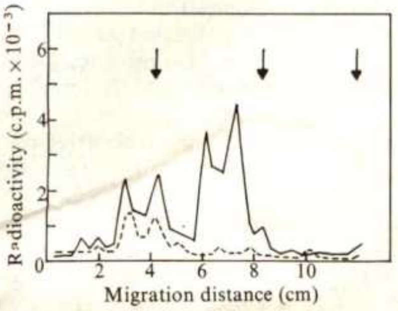

Fig. 1 SDS–polyacrylamide gel electrophoresis in reducing conditions of Triton X-100-solubilised, ^{125}I-labelled epidermal glycoproteins reactive with a rabbit antiserum raised against highly purified HLA-D antigens (———). Normal rabbit serum served as the control (— — —). The details of the experimental procedure have been outlined elsewhere[7]. The arrows denote the migration positions (from left to right) of marker heavy and light chains of human IgG and the marker dye bromphenol blue.

The type of cell in epidermis which expresses HLA-D antigens was examined with use of the IgG-fraction of a rabbit anti-HLA-D antigen serum. Biopsies of human skin were obtained from the wrist of healthy volunteers. Thin sections were prepared from the biopsies and the presence of HLA-D antigens was assessed by an indirect immunofluorescence technique, as described in the legend of Fig. 2. Antibodies against HLA-D antigens only stained a few cells in the epidermis. Figure 2 shows that the stained cells were confined to the suprabasal part of epidermis and that they displayed dendritic processes. The appearance of the stained cells as well as their localisation in the epidermis suggest that they are not keratinocytes. To demonstrate that the latter cell type does react with antibodies, thin sections of skin biopsies were stained with antibodies against β_2-microglobulin[9]. Since β_2-microglobulin represents the common subunit of the HLA-A, -B, and -C antigens[1], which are known to be present on keratinocytes, the antibodies should stain all cells in epidermis. Figure 2 demonstrates that antibodies against β_2-microglobulin gave a bright intercellular staining of all cells in the epidermis but dermis was not stained. The specificity of the antibody binding was assessed by replacing the antisera by normal rabbit gamma globulin. Figure 2 shows that normal rabbit gamma globulin did not stain any cells in the epidermis.

Since the above data clearly demonstrate that the HLA-D antigen-bearing cells are not keratinocytes an experiment was performed to investigate whether the antigens were present on melanocytes or Langerhans cells. Advantage was taken of the fact that the epidermis of albino guinea pigs virtually lacks melanocytes[10]. We have previously shown that the antiserum cross reacts extensively with murine[8] and guinea pig Ia antigens (L.K., unpublished observation). Despite the absence of melanocytes in the guinea pig epidermis staining of the HLA-D antigens gave a similar pattern to that observed for human epidermis. This demonstrates that the melanocytes are not responsible for the presence of Ia antigens in the epidermis. Thus, the observations that keratinocytes and melanocytes do not express HLA-D antigens and the dendritic appearance of the HLA-D-bearing cells strongly suggest that it is the Langerhans cells which express these antigens.

The function of the Langerhans cells is far from understood but they may represent macrophage-like scavengers with poor phagocytic activity[11]. The recent observations that the Langer-

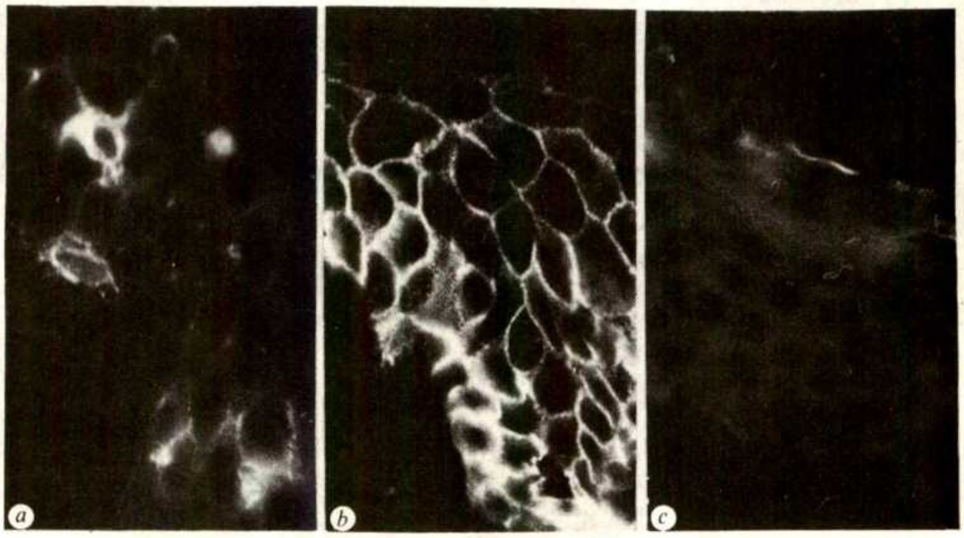

Fig. 2 Immunofluorescence of thin sections of skin biopsies after indirect staining with antibodies against HLA-D antigens (*a*) and β_2-microglobulin (*b*). In *c* normal rabbit gammaglobulin replaced the antibodies. Sections of 6 µm were prepared from skin biopsies. The air-dried sections were separately incubated with 50-µl portions of the IgG fractions from the antisera and normal rabbit serum. The IgG fractions were diluted in phosphate-buffered saline (*p*H 7.2) containing 4% of bovine serum albumin. The concentration of IgG was varied from 1 to 0.1 mg ml^{-1}. The antibodies were allowed to react with the skin sections for 30 min at room temperature in a humid atmosphere, then washed extensively in the phosphate buffer, and allowed to react with specific fluorescein–isothiocyanate-conjugated goat anti-human IgG at a working concentration of about 0.1 mg ml^{-1}. After an incubation period of 30 min and several washes the sections were mounted in 10% glycerol and examined in a Leitz Orthoplan fluorescence microscope with incident light and blue narrow band activation illuminated with a Philips × BO 75 lamp.

hans cells selectively take up antigens which are known to cause contact dermatitis[12,13] and the present finding that these cells express Ia antigens are in keeping with an immune function for the Langerhans cells. It is conceivable that the role of the Langerhans cells in contact dermatitis may be related to the following observations. It seems likely that T killer cells participate in contact dermatitis[13] but the generation of T killer cells is generally dependent on allogeneic differences involving the classical transplantation antigens as well as the Ia antigens[14]. However, chemically modified syngeneic cells may also give rise to the production of T killer cells that are directed against the individual's own transplantation antigens as well as to the foreign antigen[15]. The stimulation by the foreign antigen of the precursors of the T killer cells seems to require that the antigen is presented by macrophages expressing syngeneic Ia antigens[16]. Contact dermatitis may thus involve binding of the allergen to the Langerhans cells, which by their expression of Ia antigens may trigger antigen-specific T lymphocytes to become killer cells. These killer cells will subsequently react with any cell which expresses the syngeneic transplantation antigen together with the allergen. Since in epidermis Langerhans cells preferentially bind those antigens which are known to cause contact dermatitis the Langerhans cells should be the prime target cells. Interestingly, in contact dermatitis the number of Langerhans cells is reduced and the apposition of mononuclear cells to Langerhans cells has been observed[13].

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Cytotoxicity against tumour-associated antigens not H-2 restricted

CYTOTOXIC thymus-derived lymphocytes (T cells) can be generated both *in vivo* and *in vitro* against many different types of cell surface antigen (reviewed in ref. 1). There have been several reports that when mice were injected with non-oncogenic viruses[2–4] or when spleen lymphocytes were sensitised *in vitro* against chemically modified syngeneic cells[5], the resultant cytotoxicity was directed only against target cells which shared with the sensitising and/or effector cells in the H–2K or H–2D region of the major histocompatibility complex (MHC). Similar observations have been made with several experimental tumour systems[6–10]. This indicates that the interaction between immune cytotoxic T lymphocytes and target cells is H–2 restricted and compatibility at the H–2K or H–2D regions of the MHC is essential. But we now have data which indicate that identity at the H–2 complex (or at subregions within this complex) is not required for cytotoxic reactivity between T lymphocytes from mice immunised against murine sarcoma virus (MSV) and tumour-associated antigens on target cells. Spleen cells from immune animals could lyse either syngeneic or allogeneic tumour targets carrying the appropriate tumour-associated antigens. Thus it seems that the antigenic moiety against which the MSV-immune T cell is directed is not exclusively a modification of the H–2 antigen. Furthermore, the magnitude of the cytotoxicity against allogeneic target cells depended on the strain of animal used for immunisation.

BALB/c (H–2^d) or C57BL/6 (H–2^b) mice, 8–12 weeks old, were injected intramuscularly in the leg with a regressor strain of Moloney-MSV. Tumours appeared 7–9 d later, reached maximum size at about 14 d, and then regressed. Spleen cells were collected from the mice 12–14 d after virus inoculation—the peak of cell-mediated cytotoxicity as measured by the ^{51}Cr-release cytotoxicity assay[11,12]—and tested for their cytolytic activity in a 16–18-h ^{51}Cr release assay. The target cells were Rauscher (RLV) or Moloney (MLV) virus-induced lymphomas or chemically induced lymphomas of C57BL/6, BALB/c, A/J or DBA/2 mice (Table 1). These chemically-induced lymphomas were not susceptible to lysis by syngeneic T cells from MSV-immune mice and served as specificity controls.

Spleen cells from both C57BL/6 and BALB/c MSV-immunised mice exhibited strong cytotoxic reactivity against MLV- or RLV-induced syngeneic tumour targets as shown by the representative experiments in Tables 2 and 3. This agrees with previous reports[11,12]. But various levels of cytotoxicity were also observed against allogeneic MLV or RLV tumour target cells, with the strength of the response dependent on the strain of mouse from which effector cells were derived. C57BL/6 spleen cells showed very strong activity against tumour-associated antigens on allogeneic tumour cells. On the other hand, BALB/c effector cells were not as efficient at lysing C57BL/6 tumour targets, for although significant levels of cytotoxicity against allogeneic targets were observed in some cases (Table 2 experiment 1*b*, MBL-2 and experiment 2*b*, RBL-5), reactivity was mostly very low. Cytolysis was specific and not directed against alloantigens (Table 3). MSV-immune B6 effector cells were cytotoxic against syngeneic (H–2^b) and allogeneic (H–2^d or H–2^a) tumour targets but did not lyse allogeneic phytohaemagglutinin (PHA) blasts. Furthermore, there was no lysis against H–2 identical tumour cells which did not carry the appropriate antigen (Table 2 experiments 1*a* and 2*b*) even though these targets could be lysed by alloimmune cytotoxic effector cells (data not shown). When the effector cells were treated with anti-Thy 1.2 antibody plus complement, they could not mediate cytotoxicity, thus confirming the T-cell nature of this immune function (Table 4).

Table 1 Description of lymphoma tumour lines

	Strain of origin	H–2 type	Inducing agent
RBL-5	C57BL/6	H–2^b	RLV
MBL-2	C57BL/6	H–2^b	MLV
EL-4 (G-)	C57BL/6	H–2^b	Benzpyrene
YC8	BALB/c	H–2^d	MLV
LSTRA	BALB/c	H–2^d	MLV
MCDV-10	BALB/c	H–2^d	RLV
L1210	DBA/2	H–2^d	Methylcholanthrene
YAC	A/J	H–2^a	MLV

Direct cytotoxic reactivity against tumour-associated antigens on allogeneic tumour cells has not been described before. Herberman *et al.*[13], using a 4-h ^{51}Cr release cytotoxicity assay, saw no cross reactivity against tumour target cells of another strain. But the more sensitive cytotoxicity inhibition assay showed several of the allogeneic tissue culture lines to have

Table 2 Cytotoxic reactivity against tumour-associated antigens on syngeneic and allogeneic tumour cells

MSV immune spleen cells from	RBL–5 ($H–2^b$)	MBL–2 ($H–2^b$)	EL–4(G-) ($H–2^b$)	Target cells* YC8 ($H–2^d$)	LSTRA ($H–2^d$)	MCDV–10 ($H–2^d$)	L1210 ($H–2^d$)
Experiment 1							
(*a*) C57BL/6 ($H–2^b$)	35.9 (0.6)+	†37.0 (1.4)+	−1.1 (0.9)	36.4 (1.2)+	18.2 (1.5)+		
(*b*) BALB/c ($H–2^d$)	0.8 (0.4)	5.2 (1.4)+		19.6 (1.0)+	20.3 (1.1)+		
(*c*) BALB/c ($H–2^d$)	−2.2 (1.5)	2.2 (1.0)		17.8 (1.3)+	15.3 (1.6)+		
(*d*) BALB/c ($H–2^d$)	−2.4 (1.3)	0.3 (0.3)		39.3 (0.9)+	18.8 (1.3)+		
Experiment 2							
(*a*) C57BL/6 ($H–2^b$)	54.4 (1.2)+			29.7 (2.0)+		25.9 (0.7)+	
(*b*) BABL/c ($H–2^d$)	16.4 (1.4)+			27.2 (1.3)+		14.7 (1.5)+	
(*c*) BABL/c ($H–2^d$)	1.2 (0.9)			27.9 (1.3)+		22.9 (1.5)+	−1.7 (1.3)
(*d*) BABL/c ($H–2^d$)	0.2 (1.2)			32.8 (1.3)+		21.4 (0.8)+	

2.5×10^6 spleen cells from animals immunised 12–14 d earlier with MSV were mixed with 1.25×10^4 ^{51}Cr-labelled tumour cells in 35-mm Petri dishes and incubated for 16–18 h at 37 °C on a rocking platform. Supernatants were collected and processed as previously described[12]. Spontaneous ^{51}Cr release was usually 15–35% of the total unless otherwise indicated.

*Percentage specific release of ^{51}Cr (±s.e.) from the tumour cells was calculated according to the formula

$$\frac{\text{c.p.m. supernatant experimental} - \text{c.p.m. supernatant control}}{\text{Total c.p.m. incorporated into cells}} \times 100$$

†$P < 0.01$ as calculated by Student's *t* test compared with control.

detectable levels of antigen[13,14]. On the basis of these inhibition studies, it was concluded that the cytotoxic reactivity of spleen cells from MSV-injected C57BL/6 mice was directed against an endogeneous C-type virus-associated antigen, MEV-SA-1, and even xenogeneic target cells infected with the appropriate virus were found to carry the antigen[15,16]. In contrast, Gomard *et al.*[9] examined the interrelationship between T-lymphocyte cytotoxicity and products of the H–2 complex and concluded that recognition of antigens on the surface of tumour cells by MSV immune T lymphocytes was determined by an H–2 region product. The results of an 18-h ^{51}Cr release assay, however, confirm our previous inhibition data and demonstrate that substantial levels of cytotoxic reactivity can occur against allogeneic tumour cells (especially when effector cells from C57BL/6 MSV-immune mice are used) even though reactivity is still higher and occurs earlier against syngeneic targets (our unpublished observations). Thus in this system we see H–2 preference but not H–2 restriction. Similar observations have been made in virus-infected or hapten-modified systems where some cytotoxic reactivity was also observed against allogeneic modified target cells[17,18].

A further point should be made regarding the specificity of the cytotoxic reactivity. As noted, the antigen recognised on syngeneic tumour cells by C56BL/6 MSV-immune cells is MEV-SA-1. We have not so far identified the antigenic specificity (or specificities) in addition to MEV-SA-1 which can be recognised by these mice on the allogeneic target cells nor have we identified the antigens recognised by BALB/c MSV-immune effector cells. Nevertheless, although the mice were injected with Moloney-MSV, the cytotoxic reactivity against either syngeneic or allogeneic tumour targets did not seem (as suggested[19]) to be associated with a type-specific MLV-induced antigen, for reactivity was observed with each mouse strain against both syngeneic and allogeneic MLV- and RLV-induced lymphomas.

Our findings suggest several models for the control of the specificity of the interaction between tumour-associated antigen and T lymphocytes. First, the tumour-associated antigens induced by the oncogenic viruses in different strains of mice are similar or identical (therefore unrelated to H–2 products) and a receptor on the T lymphocyte for the H–2 of the sensitising cell enables the killer cells to interact more effectively with the target. Such a model would accord with the preference for the syngeneic targets expressed by the MSV effector cells. Second, there are several types of cell surface antigens induced by the virus, with each mouse strain, expressing unique antigens as well as common antigens; in addition, cytotoxic reactivity is stronger against the unique antigens. In such a case, there would have to be at least two subpopulations of effector T cells generated by MSV immunisation, one which reacts predominantly against the unique antigen on syngeneic cells and another which reacts with tumour-associated antigens with allogeneic cells. Third, immune response genes may be controlling the generation of the cell-mediated response and, in such a case, the animal may be able to recognise only some of the antigens induced by the virus. Therefore, although the antigens expressed on the tumour cells may have some common antigenic moieties, the response may be restricted by the ability of the host to recognise that determinant. We cannot distinguish whether one or more of these alternatives are operative in the MSV system.

Table 3 Specificity of the cytotoxic reactivity by MSV-immune lymphocytes in the 18-h ^{51}Cr release assay

Target cells	H–2 type	Effector cells* C57BL/6 ($H-2^b$) MSV immune	C57BL/6 ($H–2^b$) anti-P815 ($H–2^d$)	Baseline‡ release
RBL-5	$H–2^b$	28.7 (2.1)+	0	23.3
MBL-2	$H–2^b$	24.8 (0.7)+	0	43.6
MCDV-12	$H–2^d$	20.0 (0.7)+	41.3 (0.8)+	23.2
YAC	$H–2^a$	21.2 (1.1)+	41.4 (3.0)+	21.5
PHA blasts				
BALB/c	$H–2^d$	2.5 (1.4)	13.8 (1.7)+	40.7
DBA/2	$H–2^d$	0	18.0 (3.7)+	47.9

*See footnotes for Table 2.

‡Baseline control values were determined using normal thymocytes or non-reactive lymphocytes that gave release values which were below or not significantly different from the thymocyte control.

Table 4 Treatment of MSV-immune effector cells with anti-Thy 1.2 plus complement

C57BL/6 ($H–2^b$) MSV–immune spleen cells	Targets MBL–2 ($H–2^b$)	YC8 ($H–2^d$)
Untreated	33.8 (2.2)	11.3 (1.5)
Media control	34.4 (2.0)	12.5 (1.3)
Complement	34.0 (2.3)	11.2 (1.5)
Anti-Thy 1.2 plus complement	0 (2.3)	-0.9 (1.2)

Spleen cells from C57BL/6 mice immunised with MSV were treated twice according to the following protocol: 10^8 spleen cells were incubated with 0.6 ml of medium or anti-Thy 1.2 (final 1:2 dilution) for 30 min at 22 °C. After one wash the cells were treated with medium or with rabbit complement for 45 min at 37 °C. The cells then were washed twice with medium, resuspended to the same viable cell count, and then assayed for cytotoxicity in the 18-h ^{51}Cr release cytotoxicity assay against two different tumour targets.

Similar to our demonstration of reactivity *in vitro* of immune lymphocytes against syngeneic and allogeneic tumour targets, *in vivo* immunisation procedures with these tumour lines have shown that by immunising with an allogeneic BALB/c lymphoma (LSTRA) C57BL/6 mice could be protected from challenge with a syngeneic tumour (MBL-2)[20]. T lymphocytes have a major *in vivo* role in protection against tumour challenge[21,22] and therefore assuming that the activity detected *in vitro* accurately reflected the events taking place *in vivo*, the cytotoxicity we have observed against BALB/c tumour cells by MSV-immune C57BL/6 lymphocytes might have been anticipated.

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Relationship between β-adrenergic receptors and adenylate cyclase in HeLa cells

THE physiological actions of many hormones and neurotransmitters are mediated through activation of adenylate cyclase (ATP pyrophosphate lyase [cyclising] EC 4.6.1.1) by specific cell surface receptors for each substance. The consequent increases in intracellular cyclic AMP lead to activation of protein kinases and a host of alterations in cell physiology, depending on the type of cell stimulated[1]. Adrenergic receptors, responsive to β-hydroxylated catecholamines, were divided into α- and β-types by Ahlquist[2] using classical pharmacological techniques. The β-adrenergic effects of catecholamines occur through a receptor-mediated stimulation of adenylate cyclase activity[3]. Although β-adrenergic receptors have often been described as 'tightly coupled' to adenylate cyclase, recent evidence suggests that the receptor and the enzyme are physically discrete entities[4,5], probably the products of different genes[6]. We have found that HeLa cells contain β-adrenergic receptors and that the number of receptors per cell, but not the cellular content of adenylate cyclase, increases markedly during exposure of the cell to butyrate[7]. Here we present direct evidence that the HeLa cell β-adrenergic receptor can exist in a form either 'coupled' to or 'uncoupled' from adenylate cyclase. A model system for studies of receptor–adenylate cyclase interactions is described, and preliminary results of studies on the mechanism of coupling are reported.

In previous studies on induction of β-adrenergic receptors by short-chain fatty acids we noted that while 5 mM pentanoic acid was as effective as 5 mM butyric acid for induction of β-receptors as measured by binding of ^{3}H-alprenolol, cyclic AMP production in response to isoproterenol stimulation was only increased about one-third as much by pentanoate as by butyrate[7]. This observation suggested that the specific receptor could be induced in a form which is functionally 'uncoupled' from adenylate cyclase. The total cellular activity of adenylate cyclase did not increase with butyrate treatment; Table 1 shows basal, fluoride-stimulated, and cholera toxin-stimulated adenylate cyclase activities in homogenates of untreated and 5 mM butyrate-treated HeLa cells. The induced receptors apparently become functional by associating with pre-existing adenylate cyclase. As expected, *l*-isoproterenol-stimulated adenylate cyclase activity was higher in homogenates of 5 mM butyrate-treated cells. When induction of receptors was examined as a function of butyrate concentration, we found that while ^{3}H-alprenolol binding was fully induced by low concentrations of butyrate, 10-fold higher concentrations of the fatty acid were required for full expression of *l*-isoproterenol-stimulated cyclic AMP production, as shown in Fig. 1. In general, although higher concentrations of pentanoate were required for induction of binding, at 5 mM the same levels of activity were reached with either fatty acid; pentanoate, however, was far less effective than butyrate in eliciting catecholamine-stimulated cyclic AMP production, in agreement with our earlier observations (data not shown).

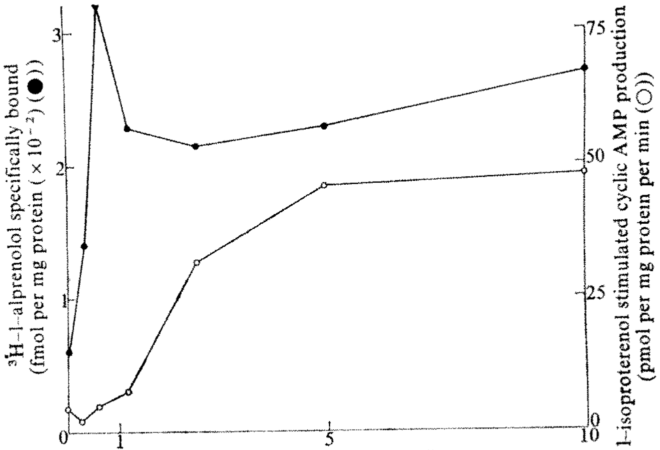

Fig. 1 Effects of various concentrations of butyrate on induction of β-adrenergic receptors and l-isoproterenol-stimulated cyclic AMP production. HeLa cells (Rhino; GIBCO) were grown as previously described[19] in minimal essential medium with non-essential amino acids and 10% foetal calf serum. 150-mm plastic tissue culture dishes were seeded at (3–4) × 10^4 cells cm^{-2} and incubated 24 h at 37 °C. The medium was replaced with fresh medium containing the indicated concentration of butyrate and the incubation continued for an additional 24 h. Dishes were washed with 10 ml phosphate-buffered saline containing 0.2% gelatin (PBS), cells collected by scraping in 10 ml PBS, and collected by centrifugation (3,000 r.p.m. for 4 min at 4 °C). Cells from one 150-mm dish were suspended for assay in 2 ml 0.9% NaCl, 50 mM Tris-HCl (*p*H 7.4) containing 5 mM theophylline and 9 mM 3-isobutyl-*l*-methylxanthine. Specific binding of ^{3}H-dihydroalprenolol and *l*-isoproterenol-stimulated cyclic AMP production were determined as previously described[7].

Time course studies indicated that butyrate induction of ^{3}H-alprenolol binding was complete by 10 h with either 0.5 mM or 5 mM butyrate (ref. 7 for 5 mM, unpublished data for 0.5 mM butyrate); the lower concentration of butyrate induced about 80% of the binding activity induced by the higher concentration. But, 0.5 mM butyrate was far less effective than 5 mM butyrate in eliciting catecholamine-stimulated cyclic AMP production (Table 1). The differential effect of the two butyrate concentrations could not be attributed to slower induction by the lower concentration.

Table 1 Cyclic AMP production in homogenates of HeLa cells after various treatments

Activity measured	Additions to medium None	 0.75 mM butyrate	 5 mM butyrate
	(pmol cyclic AMP produced per min per mg protein)		
Basal*	8.2	9.2	11.4
NaF-stimulated*	135	152	147
Isoproterenol-stimulated*	8.8	22.4	50.6
Cholera toxin-stimulated†	70	ND‡	50

*HeLa cells were incubated 24 h in medium containing the indicated concentrations of butyrate, homogenates prepared, and cyclic AMP determined as previously described[7].

†HeLa cells were incubated for 22 h in medium containing the indicated concentrations of butyrate, cholera toxin was added (4 μg ml^{-1}, 25 ml per dish) and the cells incubated an additional 2 h; homogenates were prepared and cyclic AMP measured as above. In this separate experiment the basal activity of adenylate cyclase (pmol cyclic AMP per min per mg protein) for untreated cells and 5 mM butyrate treated cells were 13.0 and 5.0 respectively.

‡ND, not determined.

An important aspect of the differential effect of the low (0.5 mM) compared with the high (5 mM) butyrate concentration is that it permits us to obtain cells with large numbers of non-functional receptors (Table 1) and, by manipulation of the tissue culture medium contents, to convert them to functional receptors; we will refer to experiments of this type as 'coupling' studies. Additional studies indicated that 0.75 mM butyrate was optimal for maximal differential between binding activity and catecholamine-stimulated cyclic AMP production. After β-receptors were induced by exposing cells to 0.75 mM butyrate, the time course was studied by replacing the initial medium with fresh medium containing 5 mM butyrate. As shown in Fig. 2, coupling begins after a brief lag and increases sharply during the next 6 h. This experimental design was used then to study the effects on coupling of certain inhibitors and medium components.

Table 2 summarises the results of several experiments in which the effects of various inhibitors or omission of certain medium components were examined separately on both the one-step induction of functional receptors and on the coupling of pre-induced receptors with adenylate cyclase. Cycloheximide, at a concentration which we have previously found to inhibit protein synthesis more than 90% in HeLa[8], completely blocked induction of functional receptors when included with 5 mM butyrate during a 24-h incubation. Surprisingly, cycloheximide caused a 90% decrease in pre-induced hormone binding after 24 h in coupling experiments, perhaps suggesting a rapid turnover of receptor molecules. Catecholamine-stimulated cyclic AMP production was less severely suppressed which may indicate that coupling of surviving receptors to adenylate cyclase does not require protein synthesis. Whether or not RNA synthesis is necessary for receptor induction or coupling cannot be determined from the current results (Table 2); our experiments with inhibitors of RNA synthesis apparently suffer from the 'superinduction' ambiguities previously reported[9].

Current models of receptor–adenylate cyclase interactions assume that functional coupling involves physical association of the components of the system and emphasise diffusion of the components in a fluid membrane[4]. Other investigators have suggested that microfilaments are involved in the modulation of certain receptors[10] and that microfilaments may constitute structural bridges between microtubules and cell surface receptors[11]. Our preliminary results with microtubule and microfilament disrupting agents (colchemid and cytochalasin B respectively) do not implicate these subcellular structures in the coupling of β-adrenergic receptors to adenylate cyclase (Table 2). It should be noted, however, that Ash and Singer[12] have shown the involvement of microfilaments in the mobility of concanavalin A receptors but could not demonstrate an effect of cytochalasin B on receptor mobility. Also Resch *et al.*[13] have reported recently that microtubule-disruptive agents do not prevent initiation of lymphocyte activation by concanavalin A binding. Concanavalin A was inhibitory for functional β-receptor induction in HeLa, while stimulating the coupling of pre-induced receptors with adenylate cyclase (Table 2). Concanavalin A binding to lymphocytes has been reported to immobilise many other receptors on the cell surface[12]. In HeLa, concanavalin A binds extensively to the cell surface but does not inhibit ^{3}H-alprenolol binding to β-adrenergic receptors (data not shown). HeLa cells remain viable for more than 24 h when glucose is omitted from the growth medium; in these conditions receptor induction is completely blocked and maintenance of pre-induced receptors is reduced by about half. Production of cyclic AMP in response to isoproterenol stimulation is greatly reduced by the omission of glucose from MEM (Table 2). These effects should be interpreted with caution since glucose-starved cells may be deficient in ATP and NADH (ref. 14). In coupling experiments, the addition of glucose to the cyclic AMP production assay mixture restored the cells' ability to produce cyclic AMP in response to the hormone (data not shown). Omission of Ca^{2+} from the medium was strongly inhibitory for receptor induction, while omission of serum had a less striking but significant effect. In contrast, the omission of either of these medium components had a stimulatory effect in coupling experiments.

These results clearly demonstrate that the β-adrenergic receptor can exist in a state in which it is functionally uncoupled from adenylate cyclase in HeLa cells. Exposure of cells to butyrate induces a striking increase in isoproterenol-stimulated adenylate cyclase activity. When high concentrations

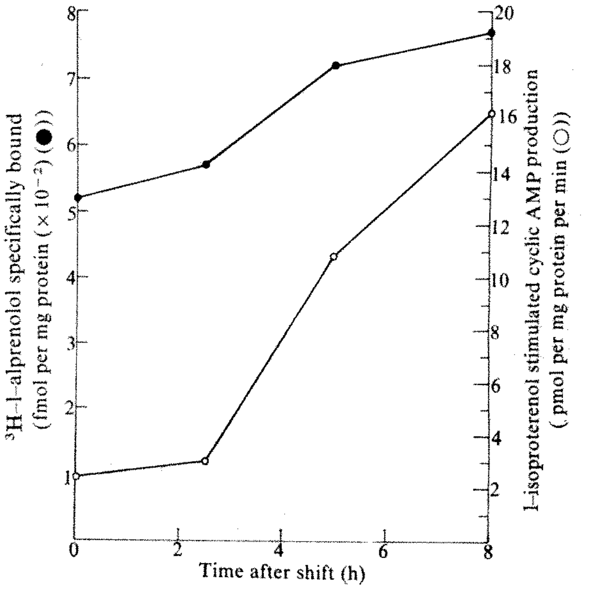

Fig. 2 Coupling of pre-induced β-adrenergic receptors with pre-existing adenylate cyclase. HeLa cells were cultured and collected and specific ^{3}H-dihydroalprenolol and *l*-isoproterenol-stimulated cyclic AMP production determined as described in the legend to Fig. 1. Cells were incubated in medium containing 0.75 mM butyrate for 20 h and transferred to fresh medium containing 5 mM butyrate at zero time.

Table 2 Effects of inhibitors and/or omission of medium components on β-adrenergic receptor induction and coupling to adenylate cyclase

Addition/omission to medium+5 mM butyrate	Induction of functional β-receptors		Coupling of β-receptors and adenylate cyclase	
	^{3}H-dihydroalprenolol bound (%)	Iso-stimulated cyclic AMP (%)	^{3}H-dihydroalprenolol bound (%)	Iso-stimulated cyclic AMP (%)
None	100	100	100	100
+ Cycloheximide (20 μg ml^{-1})	14	<2	12	41
+ Actinomycin D (1 μg ml^{-1})	55	212	31	152
+ Cordycepin (10 μg ml^{-1})	249	139	ND	ND
+ Colchemid (1 μg ml^{-1})	91	91	101	98
+ Cytochalasin B (1 μg ml^{-1})	84	91	124	126
+ Concanavalin A (25 μg ml^{-1})	43	46	144	204
− Glucose	<1	<1	39	<1
− Ca^{2+}	<1	29	164	188
− Serum	56	53	281	127

For induction of functional β-receptors, HeLa cells were incubated for 24 h in medium, the medium replaced with medium containing 5 mM butyrate and the indicated addition or omission, incubation continued for 20 h and the cells collected. For coupling studies, cells were incubated 24 h in medium and the medium replaced with medium containing 0.75 mM butyrate; after 20 h incubation this was replaced with medium containing 5 mM butyrate, incubation continued for an additional 20 h and cells collected. ^{3}H-dihydroalprenolol binding and isoproterenol-stimulated cyclic AMP production were determined as before[7]. ND, Not determined.

(>2 mM) of butyrate are used, β-receptors are induced and become functionally coupled with pre-existing adenylate cyclase molecules within 10 h. At lower concentrations of the fatty acid, the receptors increase in number but do not become functionally coupled with the enzyme; a subsequent shift to the higher butyrate concentration leads to the onset of coupling after a brief lag. This system may prove useful for the study of receptor–adenylate cyclase interactions.

The effects of short-chain fatty acids on HeLa and other mammalian cells in culture are manifold. Among the phenomena elicited by the exposure of cells to butyrate are the following: (1) The inhibition of growth and alteration of morphology in HeLa[15], Chinese hamster ovary[16], and neuroblastoma cells[17]. (2) Increases in the ganglioside G_{M3} and its biosynthetic enzyme in HeLa[18–20]. (3) Increase in alkaline phosphatase activity in HeLa[21]. (4) Increased synthesis of the β-subunit of human chorionic gonadotropin in HeLa[22]. (5) Induction of erythroid differentiation in erythroleukaemic cells[23]. (6) Induction of tyrosine hydroxylase and acetylcholinesterase activity in mouse neuroblastoma cells[17]. And (7) shape change correlated with elaboration of microfilaments and microtubules in transformed rat kidney cells[24].

In this report we have described at least two separate effects in HeLa cells: induction of β-receptor synthesis by low concentrations of butyrate and stimulation of receptor–adenylate cyclase coupling by higher concentrations.

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Thyroid hormone controls glucocorticoid action in cultured GH_1 cells

WE have demonstrated that L-triiodothyronine (T3) stimulates growth hormone synthesis three to four-fold[1–3] and inhibits prolactin secretion[1] in cultured GH_1 cells, which constitute a rat pituitary cell line. Others have shown that cortisol also stimulates growth hormone production in GH_1 cells[4]. In GH_3 cells, a closely related cell line, cortisol stimulates growth hormone production four- to eightfold and inhibits prolactin secretion approximately fourfold[5]. Thus, thyroid and glucocorticoid hormones seem to control similar growth hormone and prolactin responses in GH_1 and GH_3 cells. Studies of the effects of glucocorticoids in these cell lines[4,5], however, have used commercial serum preparations containing physiological concentrations of thyroid hormone[6]. To examine the intrinsic response of these cells to glucocorticoid, therefore, it is necessary to use medium supplemented with serum which contains very low thyroid hormone levels. Using culture medium supplemented with hypothyroid calf serum, we have examined the influence of T3 and cortisol on the induction of growth hormone synthesis in GH_1 cells. We report here that in the absence of T3, the cells seem unresponsive to glucocorticoids, whereas in its presence cortisol markedly enhanced growth hormone production. The apparent requirement of T3 for expression of the cortisol response seems independent of any influence of T3 on total cell steroid receptor levels or the nuclear-cytoplasmic distribution of the steroid receptor, and of any effect of cortisol in modulating the level of the T3 nuclear receptor.

Figure 1 illustrates a T3 dose–response study for growth hormone production determined by radioimmunoassay with and without 100 nM cortisol after a 20-h incubation. At this time the cellular response to T3 is maximal[2]. T3 alone stimulated a 7.5-fold increase, with a half-maximal response induced by 0.3 nM T3. This is essentially the same as the value we obtained by quantifying directly the rate of synthesis using ^{35}S-methionine followed by immunoprecipitation of the intracellular labelled growth hormone[3]. At each concentration of T3, 100 nM cortisol resulted in a twofold increase in growth hormone production with a half-maximal response also at 0.3 nM T3. Cortisol therefore seems to potentiate the action of T3 without altering the

T3 dose–response characteristics. In both cases, the biological dose–response relationships are in excellent agreement with the concentration of T3 (0.3 nM) that results in half-maximal occupancy of the thyroid hormone nuclear receptor in GH_1 cells[3]. This suggests that the T3–nuclear receptor complex controls a rate limiting event in the induction of growth hormone synthesis and that cortisol enhances the response initiated by T3.

Figure 2 illustrates a dose–response curve for growth hormone production as a function of cortisol concentration in the absence of T3 and in the presence of 0.3 nM and 5 nM T3, which respectively give half-maximal and full induction. Without added cortisol, the rate of growth hormone production induced by the two T3 concentrations is identical to that in the dose–response curve in Fig. 1. In the absence of T3, cortisol up to 5 μM resulted in no increase in growth hormone production. With 5 nM T3, growth hormone production was maximal with 100 nM cortisol, and decreased slightly with higher steroid concentrations, probably due to a reduction in rates of synthesis of cell protein[4,5]. At each T3 concentration, there was a half-maximal increase in growth hormone production at 18 nM cortisol, which agrees with the cortisol dose–response curve for tyrosine aminotransferase reported for hepatoma tissue culture (HTC) cells[7]. The regulation of enzyme induction by glucocorticoids in HTC cells is mediated by steroid binding to a cytosol receptor[8], followed by translocation of the steroid–receptor complex to the nucleus[9,10]. Based on the dose–response studies of the growth hormone response, it seems likely that cortisol acts by a similar steroid receptor mechanism and in some way potentiates the action of T3 which functions as the primary inducer.

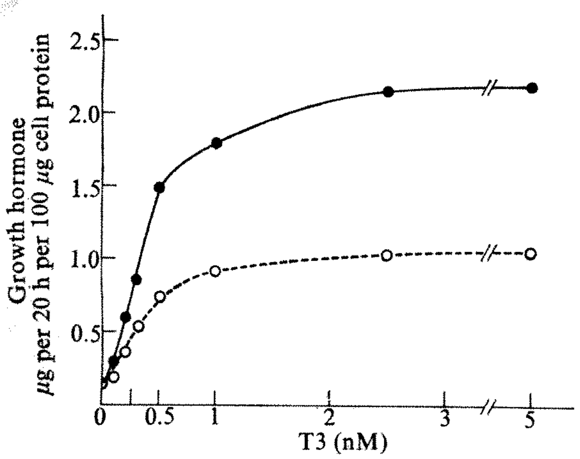

Fig. 1 Influence of cortisol on the induction of growth hormone production by T3. GH_1 cells were grown using Ham's F-10 medium supplemented with 2.5% foetal calf serum and 15% horse serum as previously described[2,3,6]. The cells (150,000) were inoculated into wells of a 24-position Falcon multiwell plate, each with a surface area of 2 cm². After 48 h at 37 °C, the medium was exchanged with Ham's F-10 medium containing 10% hypothyroid calf serum and the cells were incubated for a further 48 h. The medium was then replaced with Ham's F-10 medium containing 10% hypothyroid calf serum and T3 was added at the concentrations indicated with (●) and without (○) 100 nM cortisol. The cell cultures were incubated for a further 20 h and growth hormone was quantified in the medium by radioimmunoassay[1]. Because T3 (ref. 2) and glucocorticoids[5] have no effect on the intracellular or extracellular degradation of growth hormone, and the intracellular pools are extremely low, the level of growth hormone determined in the medium by radioimmunoassay is an indirect measurement of rates of synthesis of growth hormone during incubation[2,5]. After the medium was removed, each well was washed three times with 0.14 M NaCl at 4 °C and total cell protein was determined by the method of Lowry *et al.*[22]. Rates of production of growth hormone are expressed as μg of growth hormone produced per 20 h per 100 μg of cell protein. Each well contained approximately 60 μg of cell protein.

This is supported by studies using an anti-glucocorticoid in GH_1 cells. Samuels and Tomkins[7] identified several compounds (anti-inducers) which did not induce tyrosine aminotransferase in HTC cells but competitively inhibited the induction of the enzyme by cortisol. These findings were interpreted in terms of the interaction of these steroids with an allosteric receptor system concerned with induction of the enzyme. Two of these compounds, 17α-methyltestosterone (17αMeT) and progesterone, seem to act by inhibiting the binding of glucocorticoid to its receptor[8], and evidence indicates that anti-inducers modify mRNA accumulation[11-13]. Table 1 illustrates the effect of the anti-inducer 17αMeT on the growth hormone response induced by T3 and by T3 plus cortisol. The growth hormone response was assessed in separate experiments using radioimmunoassay[1] to measure total hormone production, and by incubating cells with ^{35}S-methionine for 15 min followed by immunoprecipitation to quantify relative rates of synthesis[2,3]. By either method, neither cortisol nor 17αMeT alone or in combination, stimulated growth hormone synthesis. In each case, T3 stimulated an increase in the growth hormone response and this was stimulated threefold further by 100 nM cortisol. 17αMeT decreased the growth hormone response in the cells incubated with T3 and cortisol to that of T3 alone, and only minimally influenced the response induced by T3 alone. Rates of synthesis of total cell protein were similar in all cases, with the exception of a slight reduction with cortisol alone or T3 plus cortisol, which is consistent with previous reports[4,5]. These results support the concept that T3 is the primary inducer of growth hormone synthesis and cortisol potentiates the T3 response by a mechanism which involves the glucocorticoid receptor.

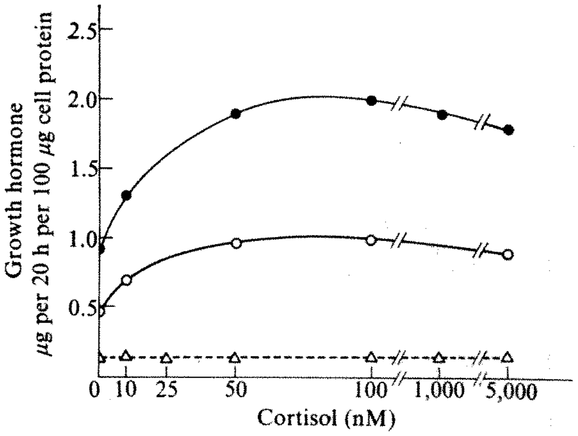

Fig. 2 Influence of T3 on cortisol induction of growth hormone production. The experiment was the same as in Fig. 1 except that the cells were incubated with cortisol without T3 (△), with 0.3 nM T3 (○) and with 5 nM T3 (●).

Growth hormone induction by T3 seems to be initiated by the association of T3 with a receptor localised in the cell nucleus[2,3,14], and seems to be explained by the accumulation of growth hormone mRNA[2]. After 24 h, 5 nM T3 results in reduction of the thyroid hormone nuclear receptor to 40% of the initial value[3]. Therefore, the potentiation by cortisol of the response to T3 might be due to prevention of T3-mediated reduction of the thyroid hormone nuclear receptors resulting in an enhanced cellular response to T3. Stimulation of growth hormone synthesis by cortisol plus T3 might also be due to a T3-mediated increase of the glucocorticoid receptors.

Table 2 shows investigation of these possibilities. As before[3], 5 nM T3 resulted in a 40% reduction in its homologous nuclear receptor population. Cortisol did not prevent receptor depletion by T3, and therefore the potentiation by cortisol of the response to T3 cannot be explained by interference in the reduction of the thyroid hormone receptor by T3.

T3 also had no effect on the level of the total glucocorticoid receptor or the nuclear-cytoplasmic distribution of the steroid

Table 1 Influence of T3, cortisol and 17αMeT on synthesis of growth hormone

Additions	Growth hormone production (radioimmunoassay, μg per 100 μg cell protein per 20 h)	Growth hormone synthesis (c.p.m. per 100 μg DNA $\times 10^{-2}$)	Total cell protein synthesis (c.p.m. per 100 μg DNA $\times 10^{-4}$)
None	0.56	688	1,051
Cortisol, 100 nM	0.50	617	800
17αMeT, 10 μM	0.48	700	1,200
Cortisol, 100 nM plus 17αMeT, 10 μM	0.49	720	1,125
T3, 5 nM	2.60	1,502	1,100
T3, 5 nM plus 17αMeT, 10 μM	2.10	1,252	1,190
T3, 5 nM plus cortisol 100 nM	7.00	3,924	960
T3, 5 nM plus cortisol, 100 nM plus 17αMeT, 10 μM	2.50	1,304	1,132

The protocol was as described in the legend to Fig. 1. Additions were as indicated. The cells were incubated for 20 h and growth hormone production was determined by radioimmunoassay of the growth hormone content of the medium. Rates of synthesis of growth hormone were estimated in a separate experiment using the same protocol as in Fig. 1, except that 1×10^6 cells were initially inoculated into 25-cm^2 Falcon T flasks[3]. After incubation for 24 h at 37 °C with compounds indicated, the cells were incubated with ^{35}S-methionine (50 μCi ml^{-1}, 635 Ci mmol^{-1}) for 15 min. Total cell protein synthesis, and growth hormone synthesis quantified by specific immunoprecipitation of intracellular labelled growth hormone, was measured as before[2,3]. Cell DNA content was determined by the method of Burton[23] and cell protein by the method of Lowry *et al.*[22]. Each point represents the mean of three determinations and each sample did not vary more than 5% from the mean.

receptor. The estimated level of glucocorticoid receptor binding sites per cell (approximately 12,000) agrees with the values reported for other cell systems[15]. The percentage of total glucocorticoid receptor identified in the nucleus, however, is approximately half of that reported for most other cell systems but is identical to that determined in glucocorticoid-sensitive lymphoma cells[16]. Therefore, the potentiation by cortisol of the response to T3 is not explained by one hormone modulating the receptor level of the other. It is possible, however, that T3 modifies a biologically relevant subset of nuclear association sites for the glucocorticoid receptor and that this is not identified by measuring the total nuclear associated glucocorticoid receptor.

Evidence indicates that glucocorticoids act at the pretranslational level to increase the rate of accumulation of mRNA[11–13,17,18]. When medium was used which was not devoid of thyroid hormone, glucocorticoids induced a concomitant increase in growth hormone production and growth hormone mRNA in GC cells, a cell line closely related to GH$_1$ cells[19,20]. This suggests that the enhancement by cortisol of the response of GH$_1$ cells to T3 occurs at a level proximal to translation. Therefore, GH$_1$ cells provide a system for the examination of the initiation and potentiation of a specific response which seems to be regulated at the level of the cell nucleus. Because glucocorticoids induce tyrosine aminotransferase in HTC cells in serum-free conditions, thyroid hormone does not seem to be important for steroid action in HTC cells[7,21]. Whether our observations with GH$_1$ cells of the interrelationship between thyroid hormone and glucocorticoid apply to other systems responsive to these hormones remains to be examined.

Table 2 Quantitfication of T3 and glucocorticoid receptors in GH$_1$ cells

Incubation	Nuclear T3 receptors (fmol per 100 μg DNA)	T3 receptor binding sites per nucleus	Glucocorticoid receptors (fmol per 100 μg DNA) Cytoplasm	Nuclei	Glucocorticoid receptor binding sites per cell
Control	180	17,800	121	35.9	12,200
T3, 5 nM	110	10,877	121	34.9	12,100
T3, 5 nM plus cortisol, 100 nM	100	9,888	—	—	—

The protocol was as described in the legend to Fig. 1 except that 3×10^6 GH$_1$ cells were inoculated into 75-cm^2 Falcon T flasks. After incubation of cells in the presence of medium containing 10% hypothyroid calf serum for 48 h, either 5 nM ^{125}I-T3 or 5 nM ^{125}I-T3 plus 100 nM of non-radioactive cortisol was added for 24 h while a parallel set of flasks served as controls. One hour before the cells were collected, 5 nM ^{125}I-T3 was added to control flasks to determine the level of receptor present in cells not exposed to T3 during the 24 h (ref. 3). The T3 bound to nuclei was determined as before[3,14]. The magnitude of nonspecific binding was determined by simultaneous incubation with a 500-fold molar excess of non-radioactive T3. This value represents less than 5% of the total T3 bound[3] and was subtracted from the total to give the values above. DNA was quantified by the method of Burton[23]. In other flasks, cells were incubated with 5 nM non-radioactive T3, while a parallel group of flasks served as controls. Glucocorticoid receptor levels were then determined by incubating GH$_1$ cells with 100 nM ^{3}H-cortisol (91 Ci mmol^{-1}) with and without a 500-fold molar excess of non-radioactive cortisol for 1.25 h at 37 °C. (ref. 24). The flasks were chilled by partially submerging them in ice for 15 min and the cell monolayers were rinsed three times with 10 ml of 4 °C 0.14 M NaCl. All further procedures were carried out between 0 and 4 °C. The cells were homogenised in buffer containing 0.25 M sucrose–20 mM Tris-HCl–1.1 mM $MgCl_2$ (*p*H 7.6 at 25 °C)[14] and the samples were centrifuged at 800*g* for 10 min and nuclei were prepared from the resultant pellets with Triton X-100 as before[3,14]. Purified nuclei were dissolved in 0.4 ml of 0.4 N NaOH and the ^{3}H-cortisol bound was determined with a liquid scintillation counter using Aquasol solution. The labelled cortisol bound in the cytoplasmic fraction was determined by incubating triplicate 1-ml fractions with 2 mg of Norit A charcoal and 0.2 mg of Dextran (Sigma, molecular weight 500,000) for 2 min followed by centrifugation at 2,000*g* for 5 min (ref. 24). The derived supernatant fractions were analysed for labelled cortisol binding after the samples had been dissolved in Aquasol. ^{3}H-cortisol binding to the nucleus or cytoplasmic fraction, bound to receptor, was calculated by subtracting the total labelled cortisol bound from that determined in the presence of a 500-fold molar excess of non-radioactive cortisol. These results represent the mean of a triplicate determination and each sample did not vary more than 8% from the mean. DNA was determined[23] on the nuclear fraction and the results are expressed as fmol bound per 100 μg of DNA. The number of receptor binding sites per cell was based on the fact that 1×10^6 cells contain 13 μg of DNA (ref. 14).

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Muscarinic antagonists block cone to horizontal cell transmission in turtle retina

As in other vertebrate retinas, the cone photoreceptor cells of the turtle are connected by complex synaptic endings with both horizontal and bipolar cells[1]. The cone endings release their transmitter continuously in the dark, and light stimulation suppresses this release[2–7]. The exact nature of the synaptic transmitter released by the cones is unknown but it is known to depolarise the horizontal cells[5,8]; to depolarise the 'hyperpolarising' bipolar cells[5,6] and to hyperpolarise the depolarising bipolar cells[5,6]. It can also be foreseen that any interference with either the release or the postsynaptic action of this transmitter will cause an hyperpolarisation of both the horizontal and the hyperpolarising bipolar cells and a depolarisation of the depolarising bipolar cells, as well as the block of the light responses of the three cell types. Lam[9] has shown that isolated cones of the turtle retina incubated in the presence of labelled precursors of acetylcholine (ACh) were able to synthesise ACh. In other species, however, assays of choline acetyltransferase activity in different layers of the retina showed a relatively low concentration of the enzyme in the outer plexiform layer (see ref. 10 for review) and ACh application to horizontal cells had no appreciable effects in carp and gekko retinas[11]. Yazulla and Schmidt[12] recently reported that binding sites for ^{125}I-labelled α-bungarotoxin (a snake venom which specifically binds to ACh nicotinic receptors) can be demonstrated by autoradiography in the retina of goldfish and turtle and that the majority of these binding sites are located in the outer plexiform layer. In an attempt to re-evaluate the possibility that ACh is the neurotransmitter liberated by cones, we studied the effects of some ACh agonists and antagonists on the cone to horizontal cell transmission. Our most interesting finding is that agents known to block the ACh muscarinic receptors block preferentially, and very probably at the post synaptic level, the synaptic transmission from the cone to the horizontal cell. Moreover, the same agents do not block the transmission from the cone to the hyperpolarising bipolar cells.

The eye was removed from the turtle, *Pseudemys scripta*, cut obliquely behind the iris and the vitreous fluid was drained. The eye cup was then mounted and fixed in a suitable chamber and perfused during the whole experiment with a bicarbonate–Ringer solution[13] (4–5 ml per min, 110 mM NaCl, 2.6 mM KCl, 2 mM $MgCl_2$, 22 mM $NaHCO_3$, 2 mM $CaCl_2$, 10 mM D-glucose) continuously bubbled with a 95% O_2+5% mixture, so as to be kept at *p*H 7.8. The drugs used were dissolved in the Ringer solution and applied to the retina through the perfusing system through a multi-way tap. Intracellular recordings were performed using single high resistance micropipettes (300–600 MΩ) filled with 4 M potassium acetate. The retina was stimulated with a double beam photostimulator, the light stimuli were images of circular apertures 50–3,600 μm in diameter. The light sources were tungsten lamps, and neutral density filters were used to reduce their intensity. The total flux density of unattenuated light at the retinal level was 6×10^3 μW cm^{-2} with wavelengths of 400–800 nm.

Retinas are very rich in acetylcholinesterase, particularly in the inner layers[10], so we thought it very unlikely that ACh could reach the outer plexiform layer without being hydrolysed. We therefore applied carbachol (an ACh analogue not hydrolysed by the esterase) to the retina and studied its effects on the L-horizontal cells. The membrane potential of L-horizontal cells varies between two extreme levels, a dark potential level and a light potential level; carbachol at high concentrations (1–5 mM) causes a depolarising shift of both the dark and light membrane potential levels, thus markedly decreasing the light responses of L-horizontal cells. This effect was reversible and disappeared after washing, but this action of carbachol was not observed in all experiments.

In spite of the irregularity of the action of this agonist, we pursued our pharmacological study by applying to the L-horizontal cells a series of ACh antagonists. The nicotinic antagonists, gallamine (flaxedil), hexamethonium and tetraethylammonium in concentrations up to 5 mM and tubocurarine at up to 1 mM neither altered the dark potential nor reduced the light responses of the horizontal cells. The muscarinic antagonists, in contrast to the nicotinic antagonists, showed a consistent blocking action. As seen in Fig. 1, application of atropine (1–5 mM) caused a progressive hyperpolarisation and, in parallel, a marked and progressive reduction of the light responses (Fig. 1*c*). Atropine also slowed down the hyperpolarising phase of the responses and caused the appearance of a small off-depolarisation (Fig. 1*d*). The responses became almost completely blocked after 15–20 min of atropine perfusion (Fig. 1*c*, *d*). The action of atropine could be reversed, but only by prolonged washing. Effects similar to those produced by atropine were produced by other muscarinic antagonists such as hyoscine and probanthine, but their blocking efficacy was lower at similar dose ranges.

The effect of other ACh agonists on L-horizontal cells was also studied. The nicotinic agonist, decamethonium (5 mM), was inactive. The muscarinic agonist, pilocarpine (1 mM), depolarised the L-horizontal cell membrane potential and reduced the amplitude of the light responses, and at higher doses, produced a mixture of antagonistic and agonistic effects. Amongst the other muscarinic agonists tested, arecoline (1–5 mM) behaved as an antagonist, blocking the responses as atropine does, and acetyl-β-methylcholine was completely ineffective.

We also explored the action of anticholinesterase agents on the L-horizontal cells. Eserine (2–5 mM) caused a depolarising shift of the dark potential which was followed by a hyperpolarisation. This latter phase was accompanied by a marked decrease of the light response, the development of a large off-depolarisation and a marked depolarising shift of the membrane potential level in the light. Analogous but milder effects were produced by similar concentrations of neostigmine and edrophonium.

The data relating to the action of atropine and other muscarinic antagonists deserve a more detailed analysis. Thus atropine was found to show some presynaptic action, since it caused both a slight hyperpolarisation of the cones (Fig. 1*a*, *b*) and a reduction of their response to light. But, the effect of atropine on the L-horizontal cells cannot be explained by this presynaptic effect alone: Fig. 1 shows that atropine reduced the cone response by at most one-third of its original amplitude, whereas the L-horizontal cell response was almost completely suppressed.

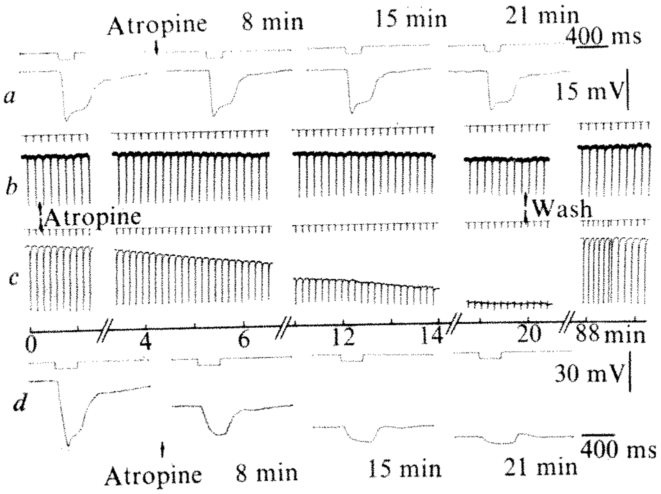

Fig. 1 Intracellular recordings from a cone (*a*, *b*) and from an L-horizontal cell (*c*, *d*) of turtle retina. The trace above each recording gives the timing of the light stimulations. Both cells were stimulated with white light (relative intensity, −1.3), the cone with a 155 μm diameter light spot whereas the spot used for the horizontal cell had a diameter of 3,600 μm. The low speed recordings in (*b*) and (*c*) have a common time base as shown below the (*c*) trace. Application of atropine 5 mM (arrows) caused in the cone (*b*) a slight hyperpolarisation and reduction of amplitude of the light response, which became diminished by one-third. In (*c*), atropine produced a hyperpolarisation of the L-horizontal cell and almost blocked the light response. After recordings (*b*) and (*c*) prolonged washing resulted in a recovery of the response of both cells. *a* and *d* correspond respectively to high speed recordings from a cone and L-horizontal cell. The light responses of both cells were recorded (from left to right) just before, and after 8, 15 and 21 min of atropine application. Note that in (*d*) the almost complete block of the horizontal cell light response and the development of an off-depolarisation.

Moreover, and more importantly, atropine was found to have little effect both on the dark potential and on the response of the hyperpolarising bipolar cells to light spots which stimulated the centre of their receptor field (that is, which activated the direct connection between the cone and the bipolar cell). This observation was confirmed in 10 different hyperpolarising bipolar cells (recognised as such by the criteria set by Schwartz[14] and Richter and Simon[15]). Figure 2*a* corresponds to a typical response of one of these bipolar cells in response to a central light spot, showing both the membrane noise decrease during the hyperpolarisation and an off-depolarisation. The response of Fig. 2*b* was recorded from the same cell after more than 25 min of atropine application, and it is evident that the response was only slightly reduced, and that the membrane noise was not altered, the small reduction of the light response amplitude being probably a consequence of the aforementioned diminution of the cone response (moreover, in two cases we observed an actual increase of the bipolar response under atropine). We conclude from this experiment that atropine does not affect the connection between the cones and the hyperpolarising bipolar cells, and therefore the effect of the drug on the L-horizontal cells cannot be presynaptic. If the transmitter release from the cones were actually decreased, the bipolar cells would have been hyperpolarised, and both a decrease of the membrane noise in the dark and a block of their hyperpolarising responses would have been observed.

From the results presented here we conclude that atropine and other muscarinic agents block the cone to L-type horizontal cell synapses by acting mainly at the postsynaptic level. It is difficult, at this stage, to relate these results to a possible transmitter role of ACh in the cone synapses. The effective concentrations of the agonists and antagonists used were very high, and we do not know if the need for such high concentrations is due to the existence of diffusion barriers or because we are dealing with nonspecific effects of muscarinic drugs which only appear at high doses. Diffusion barrier mechanisms would also account for the irregularity of effect of some of the choline derivatives as seen by us and by other workers.

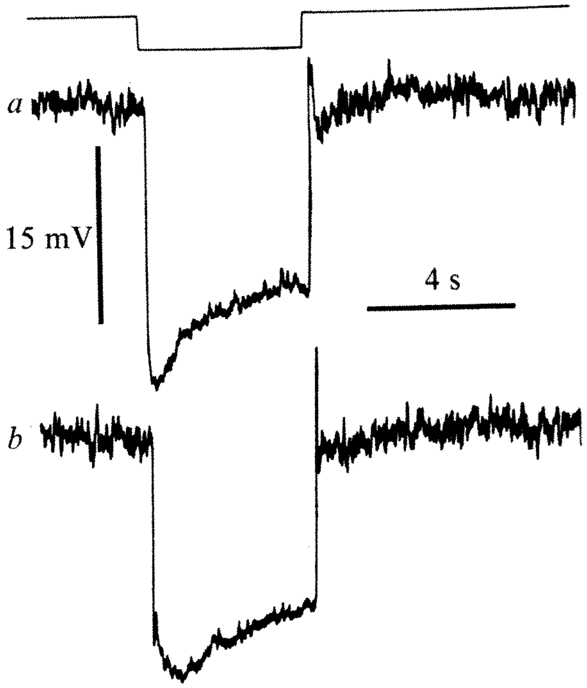

Fig. 2 *a*, Control hyperpolarising response of a hyperpolarising bipolar cell to the stimulation of the centre of its receptor field with a white light spot (diameter 600 μm, relative intensity −2.3). Note the reduction of the membrane noise during the hyperpolarisation. *b*, Response from the same cell to an analogous stimulation, recorded after applying atropine 5 mM to the preparation for 25 min. The response was slightly decreased in amplitude but no alteration in membrane noise was observed.

Finally, it is interesting to note that if ACh were the transmitter released from the cones our results imply that it should act on different receptors on the L-horizontal cells and on the hyperpolarising bipolar cells.

The main finding which we want to emphasise is that atropine blocks preferentially the synaptic responses of L-horizontal cells at the postsynaptic level. In the following paper[16] this property of atropine is used to analyse some of the known lateral cell interactions in the outer plexiform layer of the turtle retina.

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Lateral interactions in the outer plexiform layer of turtle retinas after atropine block of horizontal cells

IN the retina of the turtle and of other vertebrates, L-type horizontal cells are thought to play an interneuronal role ensuring long-distance linking between other retinal cells which synapse in the outer plexiform layer. L-type horizontal cells have been thus implicated in: (1) the feed-back loop through which peripheral stimulation evokes a depolarising synaptic potential in cones[1-3], (2) the cell network which participates in the generation of responses of opposite polarity in chromatic C-type horizontal cells[2,4,5] and (3) in the centre surround organisation of bipolar cells, where horizontal cells have been postulated to mediate the responses to peripheral stimulation[6-8]. We have shown previously[9] that atropine and other muscarinic antagonists block the cone to L-horizontal cell transmission, but do not block the cone to hyperpolarising bipolar cell transmission. Taking advantage of this pharmacological distinction, we investigated the participation of the L-horizontal cells in the above mentioned cases of lateral interaction at the outer plexiform layer. We found that atropine blocks both the feedback depolarising potential of the cones and the responses of the C-type horizontal cells, having eliminated previously to this block the opposition between the responses to different colours. At variance with these blocking actions, atropine does not block, and even increases, the depolarising surround responses of the hyperpolarising bipolar cells. These findings confirm the conclusions of previous authors that L-type horizontal cells are involved in generating both the feed back depolarising potential in the cone and the opposite responses of C-type horizontal cells to different colours. Contrary to current ideas, they demonstrate that the L-horizontal cells are not responsible for the depolarising responses of the hyperpolarising bipolar cells to the stimulation of the periphery of their receptor field.

The preparation and the general experimental procedures were similar to those described previously[9]. Atropine was used in these experiments at 5 mM. The retina was stimulated with concentric circular and annular stimuli of white or monochromatic light using apertures varying between 50 and 3600 µm in external diameter, presented either alternately or simultaneously.

Figure 1 corresponds to one of a series of experiments on the cone feedback mechanism. The recordings of Fig. 1 were obtained from a cone responding to central spot illumination with a typical 15-mV amplitude hyperpolarisation (Fig. 1*a*) and which responded to a very bright annular flash with a fast hyperpolarisation (Fig. 1*b*). The stimulation of this cone by a combination of both spot and annulus in appropriate timing (Fig. 1*c*), resulted in the appearance of a depolarising feedback potential with the characteristics described by Baylor *et al.*[1]. After bathing the retina in atropine for 15–20 min, the feedback depolarising potential of the cones disappeared completely and the annular stimuli then only evoked a small hyperpolarisation (Fig. 1*f*). It is well known that the feedback depolarisation is rather labile and that, in many cases, it may disappear spontaneously. At least in four cones studied (among them, the one recorded in Fig. 1) it was possible to observe a complete recovery of the feedback potential after removal of the atropine. These experiments on the feedback depolarising potentials confirm the existing evidence[2] on a L-horizontal cell participation in these responses.

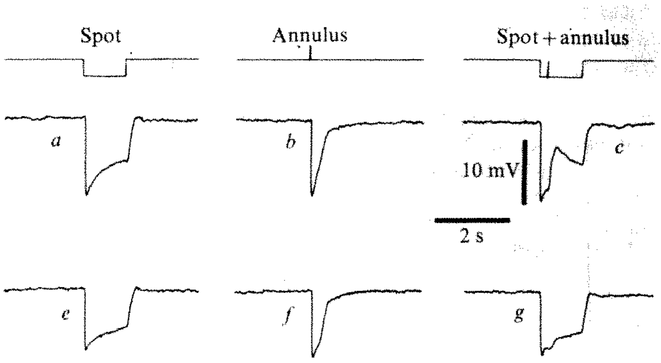

Fig. 1 Atropine effect on the feedback depolarising potential of a turtle cone. The top row of traces shows the timing and duration of the light stimuli. *a–c*, Control recordings in a cone. In (*a*) the cone responds with an hyperpolarisation to a centred spot of white light (diameter 250 µm, relative intensity −2.3). In (*b*) the same cone was stimulated with a bright annulus (internal diameter 430 µm, external diameter 3,600 µm; relative intensity −0.3) which caused a fast hyperpolarisation (which probably resulted from light scattering to the centre). The recording in (*c*) shows the appearance of a feedback depolarising potential when the annulus is presented during the centre stimulation, 180 ms after the onset of the latter. The recordings (*d–f*) were obtained from the same cone, using the same stimulation parameters, after perfusing the preparation with atropine 5 mM. Note that in (*f*) the depolarising feedback potential has been blocked and that flashing of the annulus in the same temporal relation with the centre stimulation only evokes a small hyperpolarisation due to light scattering.

Another lateral interaction mechanism in which the atropine blocking experiments support the idea of a participation of L-horizontal cells, concerns the organisation of the colour responses of the C-type horizontal cells. Figure 2 shows recordings from a green–red coded C-type horizontal cell which was alternately stimulated with large spots of red and white light. White flashes resulted in an hyperpolarisation, while red (650 nm) flashes depolarised the cell (Fig. 2*a*). Application of atropine to the preparation caused a slowly developing hyperpolarisation of the C-type horizontal cell and a decrease in the amplitude of both kinds of responses, although in the first minutes the hyperpolarising responses decreased faster than depolarising ones. After 8 min of atropine application (Fig. 2*b*) both responses were equally diminished but it could be seen that the responses to red flashes became biphasic, the depolarisation appearing then preceded by a short hyperpolarisation. Progressive blocking of the C-horizontal cells was accompanied by a reversal of the responses to red stimuli, and Fig. 2*c* shows that after 15 min of atropine perfusion both types of stimuli evoked very small hyperpolarisations. The atropine-induced reversal of the responses to red flashes can be interpreted by assuming that in normal conditions the depolarising red response masks a direct hyperpolarising response due to stimulation of the green cones, which reappears when L-horizontal cells are blocked. This finding is consistent with the interpretation[5] that the opposite responses of the red–green coded C-type horizontal cells involve L-horizontal cells interconnecting red and green cones.

The last problem of lateral interaction which we analysed with the aid of atropine is probably the most interesting one and concerns the organisation of the receptor field of the hyperpolarising bipolar cells. Figure 3 shows at left, a series of intracellular recordings from a hyperpolarising bipolar cell identified (as the group of ten we studied) by[8,10]: (1) the high sensitivity to dim light, (2) the centre-surround organisation, (3) the typical off-depolarisation after prolonged stimuli and (4), the attenuation of the membrane noise during light centre response[11]. In the experiment of Fig. 3, the hyperpolarising bipolar cell responded by a 20 mV hyperpolarisation to a centrally located spot stimulus and by a few mV depolarisation to a concentric annular flash (Fig. 3*a*). The recordings on the right side of Fig. 3 (*d, e*) were obtained from L-horizontal cells in the same preparation. The right top recording (Fig. 3*d*) is from an horizontal cell recorded before finding the bipolar cell at left and which was stimulated with a large spot with the same illumination intensity parameters used for centre stimulation of the bipolar cell. The recording of Fig. 3*e* is from one of a group of L-horizontal cells penetrated immediately after removing the pipette from the bipolar cell (that is, after an exposure of the preparation to atropine for more than 20 min). As described in the previous paper, the hyperpolarising responses of the hyperpolarising bipolar cells to a central light spot were only slightly reduced after atropine application (Fig. 3, middle and bottom left recordings), and developed complex off-phases. Moreover, the depolarising responses to peripheral stimuli were not blocked, but even increased after 20 min of atropine application (Fig. 3*c*). This observation was repeated on 10 hyperpolarising bipolar cells and in two of them the atropine treatment was maintained for more than 30 min. Since at the end of such prolonged applications of atropine, the responses of the L-horizontal cells of the same preparations were almost completely blocked (as it is the case in Fig. 3*e*), a participation of L-horizontal cells in the generation of the depolarising responses of hyperpolarising bipolar cells to the stimulation of the periphery of their receptor field can be excluded, except probably for some kind of tonic inhibition which would account for the increase of the surround responses under atropine.

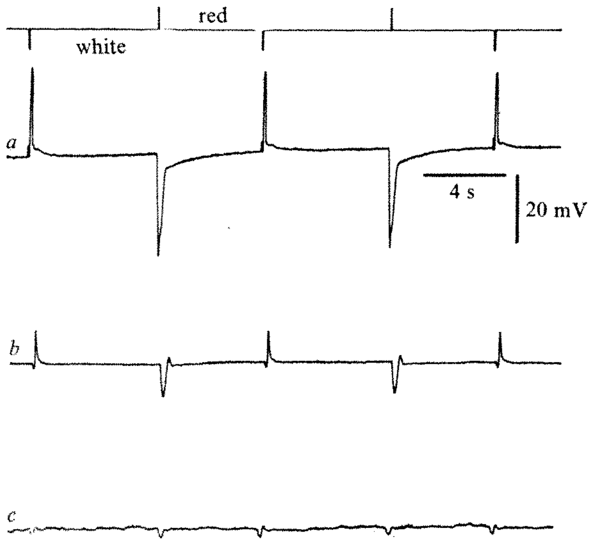

Fig. 2 Atropine effect on the responses of a green–red coded C-type horizontal cells. The top tracing marks the timing of the light stimuli, the red stimuli downwards, the white ones upwards. In (*a*) (control), the C-horizontal cell responded with depolarisations to stimulations with large spots of red light (650 nm, delivering 2.5×10^{14} photons cm^{-2} s^{-1}) and with hyperpolarisations when similar size spots of white light (relative intensity −1) were used. *b*, After applying atropine for 9 min, the C-horizontal all became hyperpolarised and both types of response seemed diminished in amplitude, but note that the hyperpolarising responses to red stimuli became preceded by a small short hyperpolarisation. *c*, After 15 min of atropine application, the responses were markedly reduced in amplitude and the red stimuli evoked only small hyperpolarising responses.

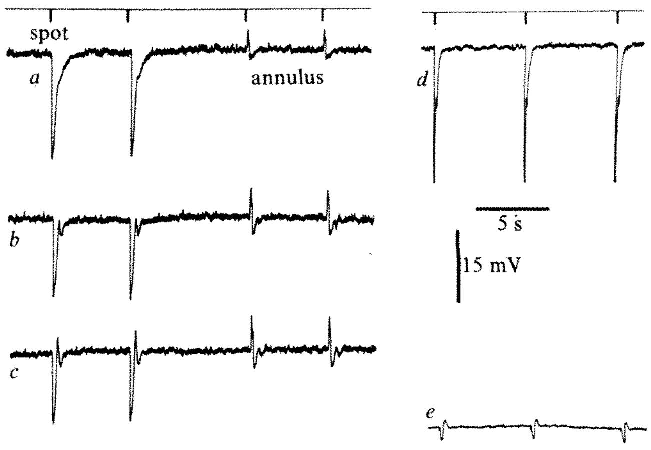

Fig. 3 At left (*a, b, c*), recordings from a hyperpolarising bipolar cell and at right (*d, e*) recordings from two different horizontal cells. The top traces on both sides mark the timing of the light stimulations. At left the two first stimuli in each row were centred light spots (diameter 380 μm, relative intensity −3) and the two following stimuli were white light annuli (internal diameter 500 μm, external diameter 3,600 μm; relative intensity −5). *a*, Control recordings of the bipolar cell response to the spot and annulus. *b*, After 10 min of atropine application, the central responses were slightly diminished and showed off-hyperpolarisations, whereas the responses to the periphery were increased in amplitude. *c*, After 20 min of atropinisation, the centre responses were not changed, except for the increasing complexity of the off responses, while the amplitude of the responses to the annulus had further increased. *d*, Recording from an L-horizontal cell impaled just before penetrating the bipolar cell at left, showing a hyperpolarising response to white light large spots (diameter 3,600 μm, relative intensity −3). The recording in (*e*) is from another L-horizontal cell penetrated at the end of the experiment, just after removing the pipette from the bipolar cell at left, while the preparation was still bathed in atropine. Notice the block of L-horizontal cell responses after more than 25 min atropine application.

It seems difficult at present to suggest what cellular elements of the retina are responsible for the antagonistic surround responses in the hyperpolarising bipolars of turtle retina. The two main possibilities, if this interaction takes place in the outer plexiform layer, are that either the periphery cones (through the long processes described by Lasansky[12] in *Pseudemys retina*) or other bipolar cells are responsible for generation of the surround responses of hyperpolarising bipolar cells.

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Membrane control of cardiac contractility

ALL myocardial cells contract in every cardiac cycle and the contractility of the heart cannot be modulated as in skeletal muscle by varying the number of active cells, so each cardiac cell must be capable of a broad range of contractile states. Such versatility could result from changes in the action potential, in the amount of Ca^{2+} released for activation of the contraction, or in the contractile proteins themselves. Phosphorylation of the inhibitory subunit of troponin seems to occur in parallel with the increase in contractility of isolated hearts perfused with catecholamines[1,2], and studies using isolated proteins indicate that the phosphorylation may be associated with a change in ATPase activity[3,4], but a direct demonstration that the functional properties of contractile proteins themselves can be altered in a manner compatible with a physiologically important regulatory mechanism has not yet been produced. We report here studies of cardiac cells with membranes that have been made hyperpermeable, in which the response of the contractile system to Ca ions, the presumed trigger for activation of contraction, may be altered markedly with respect to both the concentration of calcium required for activation and the maximum generated force by the microenvironment of the contractile proteins and by reactions involving the cell's membranes. Cyclic nucleotides may be involved in some of these regulatory reactions.

For these experiments a bundle of cardiac cells from a rat right ventricle which had been 'functionally skinned' by chelating membrane calcium with EGTA was used[5]. The sarcolemma of these cells, though highly permeable to small molecules and ions, still retains large molecules such as creatine phosphokinase and pyruvate kinase which can easily be lost when the sarcolemma is removed. Proteins like adenylate cyclase and protein kinase, which may be involved in the regulation of the contractile system and which are relatively tightly associated with structural elements of the cell[6], should therefore be present in the skinned fibre and presumably responsive to changes in concentration of small inhibitory or stimulatory cofactors such as Ca^{2+}, Mg^{2+} and ATP in the bathing medium.

The force produced at any concentration of Ca^{2+} from 10^{-9} M, a level considerably below the normal threshold for activation, to 10^{-5} M, the optimal concentration for activation, can be increased by lowering the concentration of MgATP, and the maximum Ca-activated force in 0.02 mM MgATP is about twice that produced in 5 mM ATP, the approximate intracellular concentration. In addition to an increase in force production, which may be due to either more cross bridges bound to actin at a time or more force from each cross bridge, the threshold concentration of Ca required for activation is reduced, suggesting that the affinity of troponin for Ca has been enhanced. These observations are very similar to those made by Bremel *et al.*[7], on isolated myofibrils from skeletal muscles, but comparable experiments with a mechanically skinned cardiac cell, in which the sarcolemma has been removed, have not been done at the very low concentrations of MgATP (ref. 8).

Exposure of the EGTA-skinned fibres for several minutes to 5–20 μM Ca in the presence of a very low concentration of ATP produces a marked change in the contractile properties of the fibres (Fig. 1). Force, which is normally well maintained in 5 mM ATP, falls, and this decline continues for several minutes after the ATP concentration has been returned to 5 mM, indicating that the combination of low ATP and high Ca acts through an intermediate reaction and not directly on the contractile proteins. After several minutes in 5 mM ATP the fibres stabilise, but their response to activating concentrations of Ca in 5 mM ATP is now much weaker. The extent of the inhibition, which can be almost total, depends primarily on the duration of the soak in the solution with low ATP and high Ca. The requirements for the inhibition of force generation are 1 μM Ca or more and 20 μM ATP or less, but some ATP must be present. If the membranes of the skinned fibres have been disrupted with the non-ionic detergent Brij for 45 min, a reduction of ATP to only 1–2 mM is necessary, but the Ca requirements remain unaltered. Apparently two reactions, one ATP

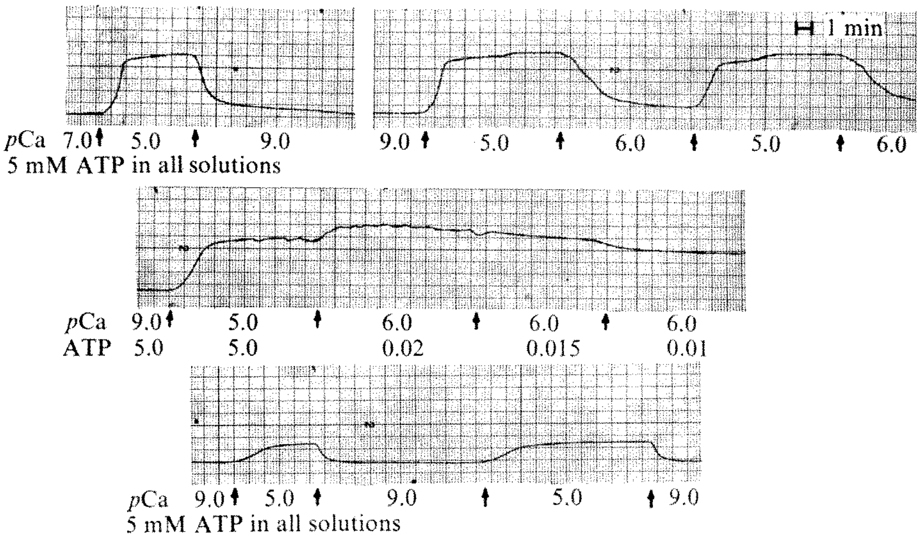

Fig. 1 Force record produced by a rat ventricular trabecula which had been functionally skinned by an overnight soak at 4 °C in a solution containing 10 mM EGTA, 4 mm MgAc$_2$, 5 mM ATP and 120 mM K propionate. The numbers under the records indicate the *p*Ca (–log[Ca^{2+}]) and the concentration of ATP in the bathing solution. The force developed in 5 mM ATP during maximal activation is very reproducible in each of the first four exposures, but when the ATP concentration is lowered to 0.01–0.02 mM the force declines. After return to 5 mM ATP the force in optimal *p*Ca is again stable, but it has been markedly reduced. The shape of the tension–Ca relation is the same after the exposure to low ATP as it was before; a uniform relative decrease in force at each concentration of Ca has occurred. In all solutions the concentration of free Mg^{2+} ions was held constant at 2 mM after taking into consideration its interactions with ATP, EGTA and creatine phosphate. The MgATP concentration was controlled by varying ATP concentration; in view of the large excess of Mg ions it was assumed that all ATP was in the form of MgATP. Ca^{2+} was buffered with 3mM EGTA and ATP with 15 mM creatine phosphate. Creatine phosphokinase, although present in high activity in the tissue, was also added with the creatine phosphate. In most experiments ionic strength was maintained by varying the concentration of KCl, but the inhibition of contractility produced by the combination of low ATP and high Ca was not particularly sensitive to ionic strength. The phenomenon occured in solutions with ionic strengths of approximately 0.7 to 1.5 times the usual value, which was approximately equivalent to 170 mM KCl. The *p*H was buffered at 7.0 with either 25 mM imidaxole or Tris at the equivalent ionic strength, and the results were independent of the specific buffer which was used. The standard test solutions with different *p*Ca that were used before and after the exposure to low ATP were identical in composition.

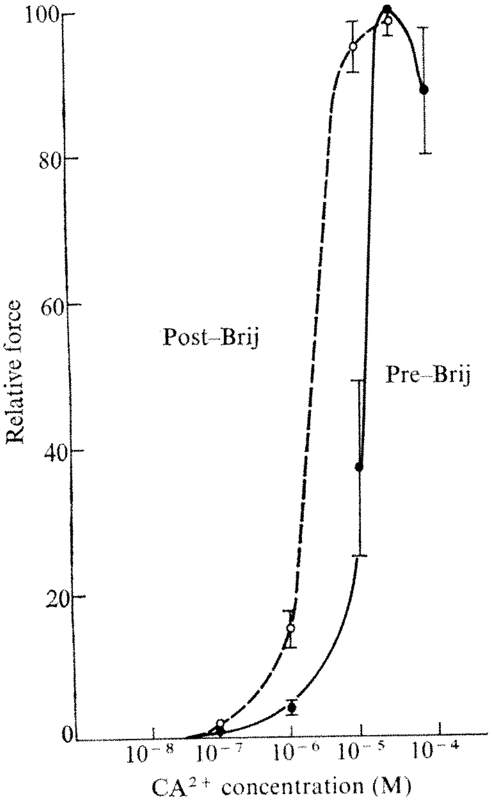

Fig. 2 The effect of the treatment of an EGTA-skinned rat ventricular bundle with Brij on the relation between force and Ca concentration. The solid curve indicates the values obtained in five preparations before Brij treatment and the interrupted curve, the values for the same muscles after 45 min in 0.5% Brij 58 followed by 60 min of washout in the standard bathing solution with no added Ca. The shift which is produced by Brij treatment has not been seen in skinned preparations where the surface membrane had been manually removed before the exposure to the detergent[9]. The Ca–tension relation found in the EGTA-skinned fibre after Brij treatment closely resembles what has been observed with the manually skinned cardiac muscle fibre[9].

dependent and requiring membranes and the other Ca dependent and not needing membranes, are involved in the change in contractility.

Exposure to Brij 58 or another detergent, Triton X-100, increases the sensitivity of the contractile system to Ca by a factor of almost 10 without altering the basic shape of the relation between Ca and force (Fig. 2). This is in contrast to the absence of any effect of Brij on the tension–Ca relation in cardiac cells from which the surface membrane had been completely removed manually[9]. A similar shift of the curve along the Ca concentration axis is produced by 5 mM theophylline or an experimental phosphodiesterase inhibitor (Squibb 20009). Apparently a nucleotide cyclase located in the membrane is involved in the regulation of the Ca sensitivity of the contractile proteins.

The effect of low ATP and high Ca on skinned fibres can be modified by the addition of sarcolemma vesicles[10] to the bathing medium. Their presence in the low ATP–high Ca solution prevents the decline in contractility, and their addition to a normal Ca-activating solution restores the original contractility to an inhibited muscle bundle for as long as the membrane vesicles remain in the bathing solution (Fig. 3). Addition of the membrane fraction to the solution bathing a non-inhibited skinned fibre causes little or no change in its contractile response to Ca. Since the preparation retains a functional diffusion barrier at its surface, the membrane vesicles are unlikely to be entering the cells and are more likely to be producing a substance which diffuses into the cytoplasm.

The capacity of the contractile system for generating force may, therefore, be controlled, at least in part, by the net result of two reactions. One reaction involving the cell membranes maintains a high capability for force generation, and the other, probably located in the myofibril, depresses the ability of the system to produce tension. The membrane reaction does not seem to involve the contractile system directly, but rather to suppress either the synthesis of an inhibitory molecule or the interaction of an inhibitory molecule with the contractile proteins. In view of the importance of the sarcolemma and the apparent inhibition by ATP concentrations below 20 μM, the first reaction may be associated with the production of cyclic AMP and the requirement of the second reaction for ATP

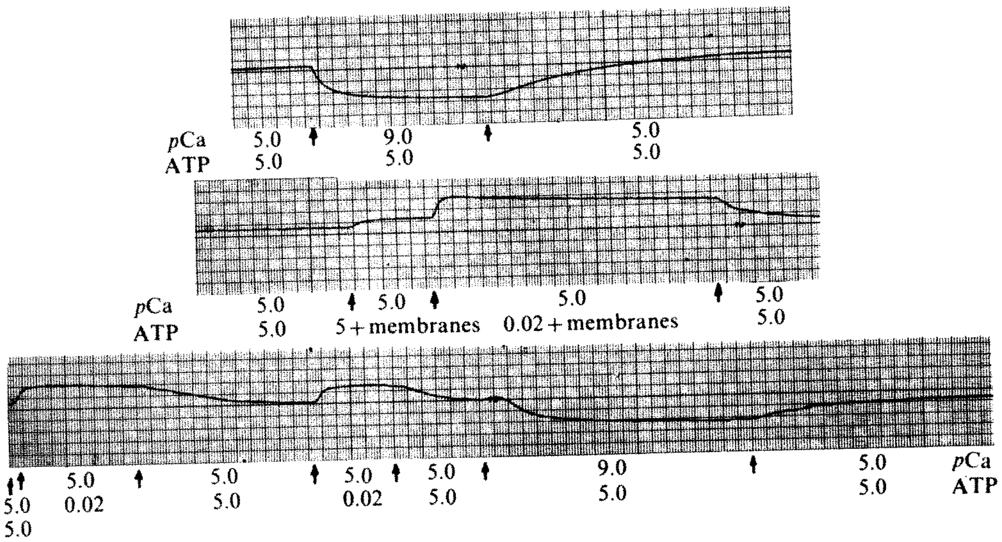

Fig. 3 The presence of isolated sarcolemmal vesicles in the bathing medium protects the EGTA-skinned rat ventricular cells from the negative inotropic effect of an exposure to the combination of low ATP and high Ca. Numbers under the force record indicate *p*Ca and mM ATP. Force in optimal Ca and 5 mM ATP is reproducible. The exposure of the tissue to *p*Ca 5.0 and 20 μM ATP does not inhibit the subsequent ability of the tissue to generate force on *p*Ca of 5.0 when sarcolemma vesicles (prepared according to Lefkowitz and Haber[10]) are present in the bathing medium. Since the membranes which were added did not show any Ca-activated ATPase, major contamination with sarcoplasmic reticulum is unlikely. ATP concentration was buffered with 15 mM creatine phosphate, which was at least 5 mM in excess of what was necessary to prevent any detectable sign of ATP limitation. Direct measurements of the bathing solution showed that the presence of the membranes caused a decline in creatine phosphate of only 1.5 mM every 30 min. The presence of 15 mM creatine phosphate at the beginning of a force measurement satisfactorily maintained the ATP concentration. The membrane fraction in the bathing solution represented an approximately 100-fold dilution of the concentration of membranes in the original ventricle, and consequently, its presence would contribute very little to the ionic strength or osmolarity of the bathing solution. The temporary protection from the presence of the membranes disappears with their removal as shown in the record, but in the absence of an exposure to low ATP without membranes, tension is well maintained for several hours after the exposure to low ATP with membranes. The small rise in force when membranes were added to the bathing solution was sometimes seen. It was completely reversible and was probably due to a temporary reversal of a small amount of existing inhibition of contractility.

suggests a Ca-regulated phosphorylation[11]. Experimentally, however, the addition of cyclic AMP or cyclic GMP alone has failed to reproduce the effect of the low ATP–high Ca solution, the membrane fraction or Brij treatment. If our inferences about their involvement are valid, the cyclic nucleotides can be active only in very specific conditions, and alteration of contractile proteins is controlled by a pattern of molecules and not by a single molecular species. The sensitivity of the potentially responsive fraction of the contractile proteins to Ca is also regulated by the sarcolemma, possibly through a cyclic nucleotide controlled phosphorylation of the contractile proteins[4].

The sensitivity of the contractile system to the concentration of ATP may represent a protective mechanism available to the cell for conserving energy for life sustaining reactions when the supply of oxygen or substrate is deficient. It would constitute a mechanism whereby the work of the tissue could be adjusted to its energy supply.

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Postsynaptic effects of a multiaction giant interneurone on identified snail neurones

A FEW examples of identifiable neurones which have widespread monosynaptic and multi-action effects on large numbers of postsynaptic cells have been found in gastropod molluscs[1–3]. Such neurones are of considerable interest to neurophysiologists attempting to understand how the electrical activity of large networks of nerve cells can be integrated. We report here that the right pedal giant neurone (RPGN) of the pond snail, *Lymnaea stagnalis* (L.), is an interneurone with monosynaptic connections with many previously identified giant cells[4] and cell clusters[5] situated on the visceral and right parietal ganglia of *Lymnaea.* The RPGN mediates excitation on some cells, inhibition on others and biphasic effects (excitation followed by inhibition) on yet other cells.

Isolated brains from 1–9-g specimens of *L. stagnalis* were prepared for electrophysiological recording as previously described[5], except that the commissure between the cerebral ganglia was cut and the ganglia spread apart. This procedure facilitated simultaneous electrophysiological recording from the RPGN in the right pedal ganglion and from its follower cells on the dorsal surfaces of the visceral and right parietal ganglia. Intracellular recordings were obtained from neuronal somata using conventional recording methods and 10–20-MΩ K_2SO_4 filled microelectrodes. Preparation of electrodes was greatly facilitated by use of triangular cross-section Pyrex electrode glass which allowed rapid, reliable filling of microelectrodes with electrolyte without previous addition of glass fibres.

The RPGN lies on the postero-ventral surface of the right pedal ganglion, medial to the statocyst and has a major soma diameter within the range 120–240 µm in different snails. The positions of the cell bodies of neurones innervated by the RPGN are shown in Fig. 1. RPGN action potentials evoked by intracellular injection of depolarising current pulses via the recording electrode result in excitatory postsynaptic potentials (e.p.s.p.s) in A group (Fig. 2*a*), the H cells (Fig. 2*e*) and K cells (Fig. 2*b, c, d*), inhibitory postsynaptic potentials (i.p.s.p.s) in the J cells (Fig. 2*b, c, d*) and VD2, VD3 (not shown) and a biphasic response (excitation followed by prolonged inhibition) in G group (Fig. 2*i, j*).

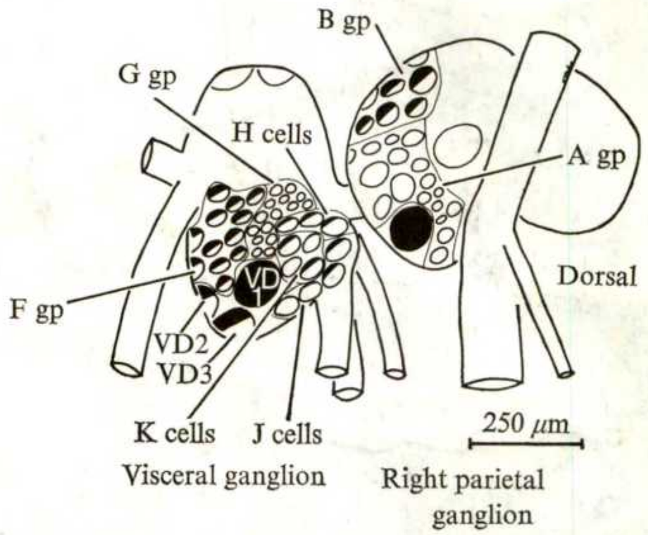

Fig. 1 Diagram of the visceral and right parietal ganglia of *Lymnaea* from the dorsal aspect. The cell body positions of several giant cells and cell clusters are indicated. Most of the neurone somata are orange in colour (unshaded), but some are pale (part shaded) and others white (filled), probably due to the light-scattering properties of small granules present in the cytoplasm. The H, J and K cells are difficult to separate from one another by appearance alone and do not necessarily lie in discrete clusters, but G group is a distinct cluster of small orange cells of 30–50 µm soma diameter. The cell bodies of A group are of variable size, but have similar properties to one another. The somata of the giant cells VD2 and VD3 (visceral dorsal cells 2 and 3) usually lie on the dorsal surface, but may sometimes be found ventrally along with the soma of VD1. The cell bodies of F group and B group spread on to the ventral surfaces of the visceral and right parietal ganglion respectively. Neither they nor VD1 receive inputs from the RPGN, but they share some common inputs with it and with the cells it innervates.

Several lines of evidence suggest that these connections are monosynaptic:

(1) One postsynaptic potential per spike could be evoked up to the maximum frequency of firing of the RPGN (about 10 Hz) (Fig. 2*a, b*).

(2) The delay between the onset of the spike in the RPGN and the start of the postsynaptic response was constant for a particular neurone (20–25 ms for e.p.s.p.s, Fig. 2*a* and *b*) even after treatment in a solution containing five times (20 mM) the usual Ca^{2+} concentration (Fig. 2*f, g, h*). The delay was more difficult to determine for i.p.s.p.s because of their slow rise time.

(3) Bathing the preparation for up to 2 h in this high Ca^{2+} saline failed to block excitatory (Fig. 2*e, f, h*) inhibitory (Fig. 2*g*) or biphasic effects. High Ca^{2+} saline would be expected to raise the threshold of any interneurone lying between the RPGN and a follower cell, thus causing synaptic block[7].

(4) Iontophoretic injection of tetraethylammonium (TEA) into the RPGN via a recording electrode containing 1 M TEA bromide resulted in a prolonged action potential and a corresponding increase in the duration of the postsynaptic response (shown for a G group biphasic response in Fig. 2*i, j*). We presume that injected TEA has the effect of blocking the delayed increase in K^+ conductance during the action potential[8], thereby prolonging the release of transmitter, and increasing the duration of the postsynaptic response in the case of a monosynaptic connection. On the basis of this evidence it seems reasonable to summarise the connections of the RPGN with its follower cells as shown in Fig. 2*k*. Further inputs common to the RPGN and its follower cells are summarised elsewhere[6].

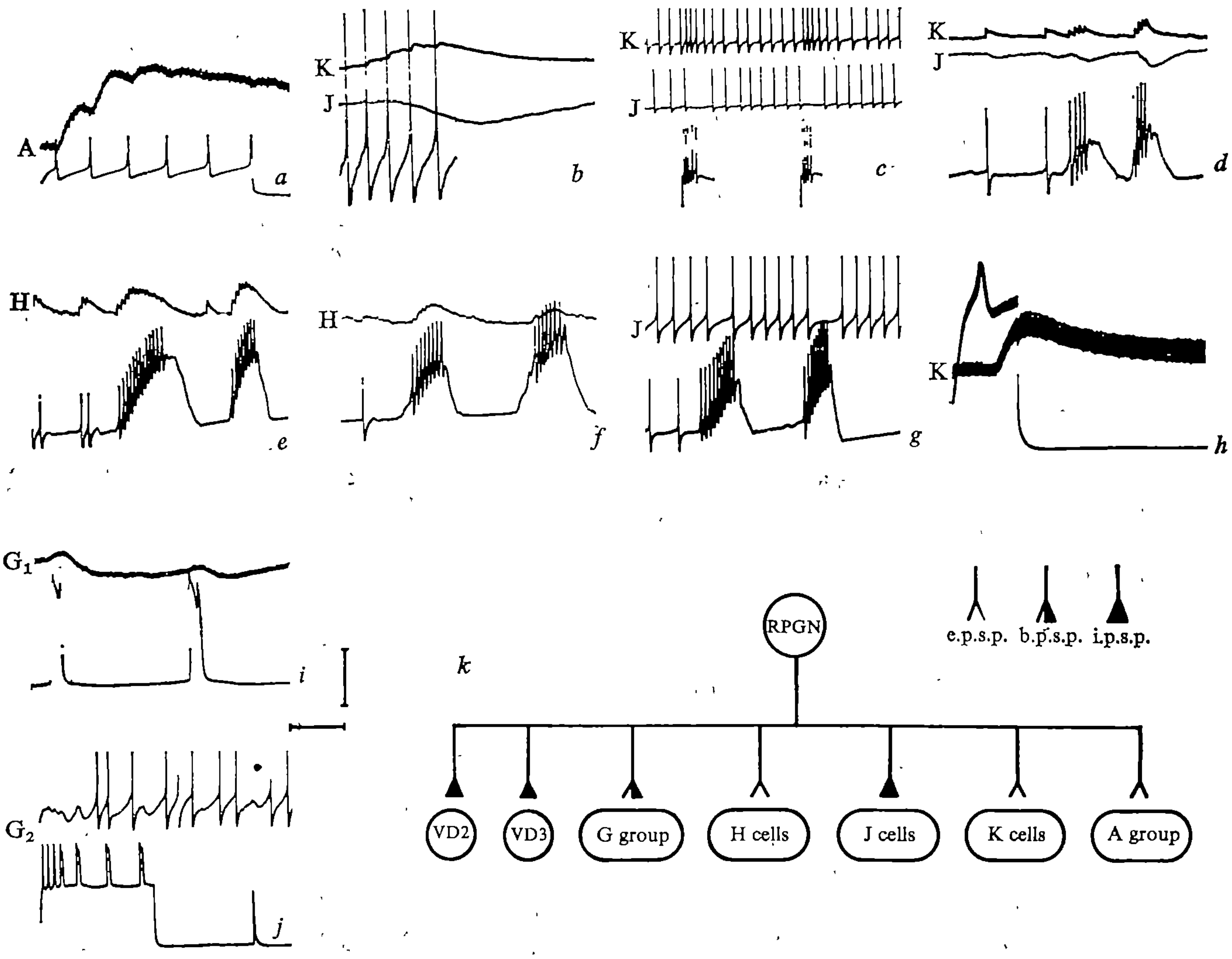

Fig. 2 Monosynaptic connections between the RPGN and its follower cells. The RPGN is on the lower trace in all cases. Other traces are from follower cells of the RPGN labelled with capital letters in accordance with Figs 1 and 2*k*. Excitatory inputs are received by the cells of A group (*a*), the K cells (*b*, *c*, *d*) and H cells (*e*), whilst i.p.s.ps are received by the J cells (*b*, *c*, *d*) and biphasic p.s.ps (b.p.s.ps) are found in the cells of G group (*i*, *j*). To demonstrate the 1:1 relationship and constant latency between spike and p.s.p. it is necessary to hyperpolarise the follower cells (*a*, *b*, *d*, *e*) by 5–15 mV since at the follower cells' normal firing level these effects cannot be clearly seen (*c*). The RPGN follower cell connections are not blocked by high Ca^{2+} saline (*f*, *g*, *h*). *f*, The same H cell as in (*e*) shown after soaking the preparation for 17 min in high Ca^{2+} saline. The e.p.s.ps are diminished in amplitude since increased Ca^{2+} levels cause a depolarisation of the G cells rather than a hyperpolarisation. cell RPGN spikes are increased in amplitude since they are partially Ca^{2+} dependent. *g*, High Ca^{2+} saline fails to block inhibition of a J cell by the RPGN; *h*, 20 repetitions of a spike in the RPGN, evoked by a 100-ms pulse at 0.5 Hz, cause a stable e.p.s.p. in a K cell after immersion in high Ca^{2+} saline for 15 min. The effects of TEA on the RPGN spike and on the b.p.s.ps recorded in two different G cells from the same preparation are shown in *i* and *j*. The b.p.s.ps are considerably enhanced and have durations in excess of 1.2 s compared with control durations of 0.5–0.7 s. In normal conditions the initial excitatory phase is usually very small or even suppressed. *i*, G_1, 310 min after the start of iontophoretic injection of the RPGN. *j*, G_2, 330 min after the start of TEA injection (0.5-s pulses at 1 Hz). *k*, Summary diagram of the monosynaptic connections of the RPGN. Time calibrations: *a*, 0.4 s; *b*, 0.8 s; *c*, *d*, 8 s; *e*, *f*, 2 s; *g*, 4 s; *h*, 0.1 s; *i*, 1 s; *j*, 4 s. Voltage calibrations: *a*, upper 4 mV, lower 100 mV; *b*, all traces 20 mV; *c*, all traces 80 mV; *d*, upper 20 mV, middle and lower 40 mV; *e*, *f*, upper 20 mV, lower 100 mV; *g*, upper 40 mV, lower 100 mV; *h*, upper 10 mV, lower 80 mV; *i*, upper 10 mV, lower 40 mV; *j*, upper 20 mV, lower 100 mV.

Sakharov[4] has demonstrated that the RPGN of *Lymnaea* is catecholaminergic and Kiss[10] indicated that several cells in the visceral and right parietal ganglia respond to iontophoretically applied dopamine with either depolarising or hyperpolarising shifts in membrane potential. Although the precise identity of these cells is as yet unclear they occur in similar areas of the ganglia to those described here and presumably correspond to some of the cells we describe. Thus the RPGN is most probably dopaminergic and therefore homologous with another multi-action neurone making widespread monosynaptic connections, the giant dopamine-containing cell (GDC) of *Planorbis corneus*[3]. The brain of *Planorbis* (a sinistral pulmonate) is a mirror image of that of *Lymnaea*. Thus the GDC lies on the left pedal ganglion and its follower cells lie on the visceral and left parietal ganglia. Unfortunately, none of the follower cells in *Planorbis* has been identified and it is not yet possible to say whether they correspond with the identified neurones we have described in *Lymnaea*.

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Spontaneous release of transmitter from 'repressed' nerve terminals in axolotl muscle

DENERVATION of the limb of a lower vertebrate is followed by an ordered reinnervation, so that the normal pattern of muscle use is restored[1]. Higher vertebrates, however, seem to be much less selective in their reinnervation[2]. There is no absolute restriction on inappropriate reinnervation in lower vertebrates[3]; the return of normal function is due, at least in part, to multiple reinnervation of muscle fibres by different nerves, followed by a repression of function of less appropriate contacts[4]. Yip and Dennis[5] have shown that the repression of function in inappropriate nerve–muscle junctions in the newt, *Notopthalmus viridescens*, involves a reduction in the number of quanta of synaptic transmitter released by each nerve impulse. If the initiating event in this process is reduction or loss of the ability of inappropriate nerve terminals to release transmitter[6,7], there should be evidence of the continued presence of these terminals even after transmission from the inappropriate nerve has been repressed. In this paper we present such evidence as a result of our studies in the axolotl.

Sectioning the anterior nerve to the supracorocoideus muscle of axolotls (*Ambystoma mexicanum*) induces the posterior nerve to sprout and innervate muscle fibres normally activated by the anterior nerve. This inappropriate innervation is subsequently repressed when the anterior nerve regenerates and reinnervates its normal territory[8] (Fig. 1). In our experiments, the anterior nerve was sectioned initially and then again, 20–100 d after the first cut, so as to cause the newly restored anterior nerve terminals to degenerate once again. Nerve–muscle transmission from anterior nerve terminals failed about 24–30 h (December–January) or 48–54 h (May–June) after this second section (similar seasonal differences in the time of survival of axotomised nerve terminals occur in frogs[9,10]). Shortly after this failure, intracellular recording revealed low frequency miniature endplate potentials (m.e.p.ps) in regions of the muscle where repressed nerve terminals were anticipated to exist (Fig. 1*d*), and not elsewhere (Fig. 2). These m.e.p.ps were not seen if both anterior and posterior nerves were sectioned. (Thirty-six cells were examined in two muscles. Muscles from the contralateral limbs were given the same initial operation but the posterior nerves were left intact in the second operation. Eight of 24 cells in the contralateral muscles had the low frequency m.e.p.ps.) Stimulation of the intact posterior nerve evoked no responses in muscle fibres exhibiting these low frequency m.e.p.ps.

This population of m.e.p.ps had properties distinguishing them from those recorded at degenerating terminals, those seen at developing nerve–muscle junctions at the stage before nerve–muscle transmission has developed[10], and also from those caused by ACh release from denervated Schwann cells a few days after nerve–muscle transmission has failed[11]. Degenerating anterior nerve terminals continued to release m.e.p.ps for 1–4 h after nerve-evoked transmitter release had failed and they occurred at multiple sites along the length of each muscle fibre, whereas the low frequency m.e.p.ps which remained after the degenerating terminals had ceased to release transmitter usually occurred at a single site near the distal end of a muscle fibre; their further characteristics are listed in Table 1.

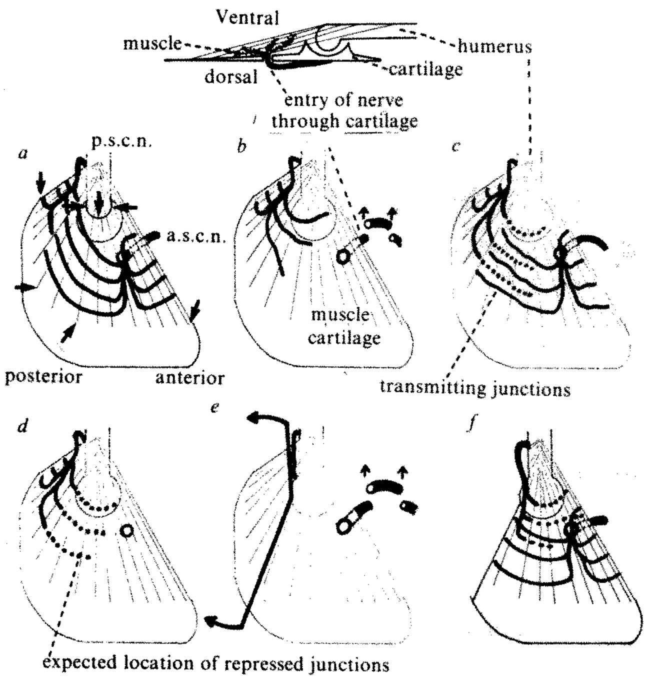

Fig. 1 Experimental scheme to locate repressed nerve–muscle junctions. Top, transverse section view through supracorocoideus muscle and cartilage; *a–f*, ventral views. The supracorocoideus muscle in the shoulder girdle of the axolotl has two separate zones of innervation (*a*). Cutting the anterior nerve (a.s.c.n.) provokes the posterior nerve (p.s.c.m.) to extend its transmission zone into more anterior regions of the muscle (*b*). Later, (*c*), the anterior nerve regenerates and resumes control of the large anterior zone of the muscle, and transmission to this zone by the posterior nerve (dotted lines) is repressed[8]. We cut the anterior nerve a second time, and shortly after its degenerating terminals had ceased to release transmitter, explored the muscle for evidence of repressed nerve–muscle junctions (*d*). In a second series of experiments (*e*, *f*) the whole posterior zone of the muscle was removed at the time of anterior nerve section so that any contacts between the posterior nerve and the muscle seen at a later time could be judged inappropriate. Supracorocoideus muscle fibres are multiply innervated by axons from one or the other nerve, and respond to nerve stimulation with action potentials. For microelectrode recording much of the cartilage was removed and the muscle approached from the dorsal (cartilage) side. The reference points marked by arrows (*a*) were mapped for each muscle using a 20× water immersion objective and the microscope stage micrometer so that relative positions of recording sites in muscles of different sizes could be compared by transferring these coordinates to a common schematic. The axolotls used in this work were all from the same mating and hatched in September 1975.

Lanthanum ions increase m.e.p.p. frequency at normal and developing nerve–muscle junctions[10,12]. In control experiments, using normal muscles, 0.5 mM $LaCl_3$ caused a 10–100-fold increase in m.e.p.p. frequency within 30 s of its addition to the perfusion system. Most applications to experimental preparations were of 2 mM $LaCl_3$, and in about half 10 mM HEPES buffer (*p*H 7.2) was added to avoid problems of excess acidity or precipitation of La^{3+}. Twenty-three muscle fibres with low frequency m.e.p.ps seen shortly after failure of anterior nerve transmission were examined for 2 min or more during perfusion with La^{3+} Ringer with a resolution of recording of 0.2 mV or better. In 19 fibres there was no change in m.e.p.p. frequency (less than twofold increase or decrease) during application of the La^{3+}. Two fibres responded with a large increase in m.e.p.p. frequency. In two more there was a slowly developing increase after an initial latent period; for example, control frequency 5 per 30 s; La^{3+} induced, 7, 6, 10, 30, and 51 per 30 s respectively.

Depolarisation of normal or developing nerve–muscle junctions by adding KCl to the bathing medium causes a prompt and maintained increase in m.e.p.p. frequency[10]. In control experiments with normal muscles, KCl concentrations of 15 or 17.5 mM caused an increase in m.e.p.p. frequency at least tenfold within seconds of its addition to the bath, whereas muscle fibres were not so depolarised that m.e.p.ps were

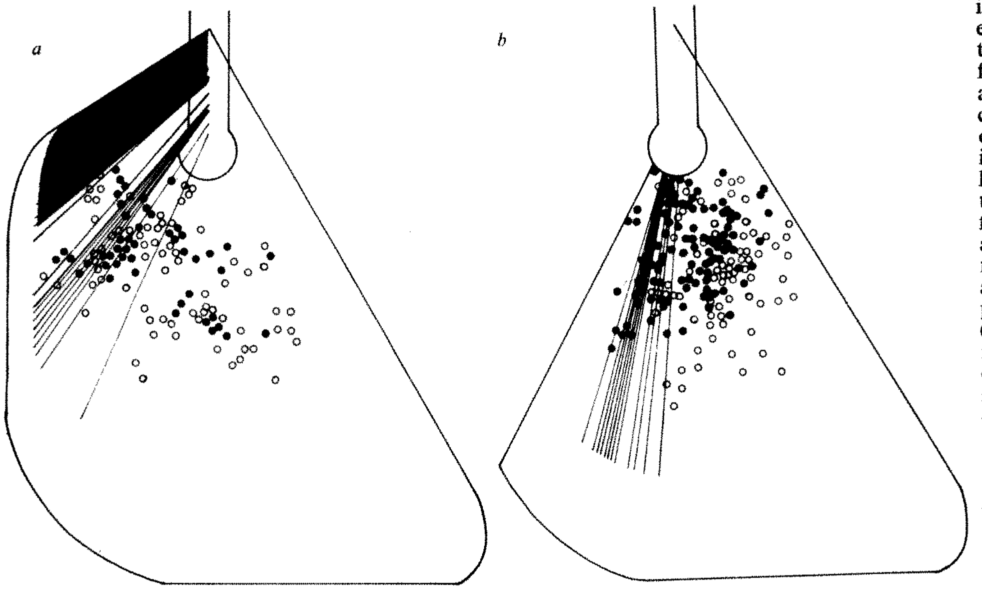

Fig. 2*a* Posterior nerve innervation zone and location of low frequency m.e.p.ps in supracorocoideus muscles following expansion and retraction of the posterior nerve innervation zone. Records from both left and right muscles from animals ranging in length from 5 to 13 cm have been placed on a common diagram using the reference points illustrated in Fig. 1*a*. ●, Fibres with low frequency m.e.p.ps and no response to posterior nerve stimulation; ○, fibres with no m.e.p.ps over a period of at least 2 min stable recording with resolution 0.2 mV or better. Shaded area, fibres contracting in response to posterior nerve stimulation in normal (control) muscles; /, borders of posterior nerve-induced contraction in individual experimental muscles. The diagram summarises results from 16 muscles examined 20–52 d after the initial section of the anterior nerve (in seven muscles the nerve was resectioned 3 weeks after the initial operation, and the muscles examined 2–3 weeks later). The anterior nerve was again sectioned 24–96 h before recording from all of the muscles. Degenerating anterior nerve terminals ceased to release transmitter 24–30 h after nerve section in these animals. Low frequency m.e.p.ps with the special properties described in the text could be identified only anterior to the border of posterior nerve–muscle transmission. This border had retracted to within its normal limits in less than half the muscles examined in this series. These experiments were carried out during January and February 1976. *b*, Nerve–muscle transmission and low frequency m.e.p.ps in preparations with the posterior portion of muscle removed at the time of anterior nerve section. Symbols as in *a*. This is a summary of results from 16 preparations examined 3 months after the initial operation, and 29–120 h after second section of the anterior nerve. These animals ranged in size from 14 to 19 cm, so anterior nerve regeneration generally took longer than in the muscles illustrated in *a*. The experiments were carried out during May and June 1976. In these experiments all muscle fibres became reinnervated by the anterior nerve, but 12 of 16 muscles retained a zone of posterior nerve innervation so that dually innervated muscle fibres occured near the posterior border of many muscles.

difficult to see. We examined 17 junctions with low frequency m.e.p.ps seen shortly after failure of anterior nerve transmission. In 14 of these there was no change in m.e.p.p. frequency (less than twofold increase or decrease) during at least 1 min of muscle depolarisation caused by perfusion with high K^+ Ringer (the muscle cell depolarisation served to monitor the effective presence of the K^+). In two fibres there was a prompt and large increase in m.e.p.p. frequency and in one fibre it began to increase 2 min after addition of the K^+ (control frequency 4 min^{-1}; K^+, 4 min^{-1}, 7 min^{-1}, 25 min^{-1}).

Slowly developing increases in m.e.p.p. frequency in response to La^{3+} or K^+ perfusion were more commonly seen when more time elapsed after anterior nerve section. For example, in muscles examined 5 d after nerve section five fibres treated with La^{3+} showed no change in frequency during the first 2 min of perfusion, but increased an average of six times during the third minute. Similarly, three fibres treated with high K^+ showed a sixfold increase in m.e.p.p. frequency during the third minute. Also, at this time, a few fibres were found where posterior nerve stimulation intermittently evoked small e.p.ps. Genat and Mark[8] found that transmission at repressed junctions in axolotl supracorocoideus muscle began to return 7 d after section of the regenerated anterior nerve. The slight restoration of sensitivity of m.e.p.p. frequency to La^{3+} or K^+ seen 5 d after anterior nerve section may mark a return towards normal function.

Increased tonicity raises m.e.p.p. frequency at normal and developing nerve–muscle junctions[13,14]. Twenty-one fibres with low frequency m.e.p.ps were tested by application of Ringer solution made hypertonic by addition of 55 mM Na_2SO_4. In 20 of these fibres m.e.p.p. frequency increased at least fivefold. Seven fibres were tested with La^{3+} and K^+ in addition; mean m.e.p.p. frequencies were: control, 10 min^{-1}; K^+, 14 min^{-1}; La^{3+}, 8 min^{-1}; hypertonic, 145 min^{-1}.

Botulinum toxin blocks nerve-evoked transmitter release and greatly reduces spontaneous m.e.p.p. frequency without affecting synaptic structure or propagation of the presynaptic action potential[15]. We treated normal axolotl muscles with large doses of type D botulinum toxin and found that the resulting low frequency m.e.p.ps had properties similar to those seen following repression of inappropriate nerve terminals. Seven botulinised fibres examined in normal Ringer had a mean m.e.p.p. frequency (±s.e. of the mean) of 0.10 ± 0.04 min^{-1}; in hypertonic Ringer 9.9 ± 5.8 min^{-1}; and in KCl Ringer 0.14 ± 0.04 min^{-1}.

The low frequency m.e.p.ps seen in the experimental muscles resembled those released from Schwann cells at denervated endplates in that their frequency was unaffected by treatment

Table 1 Properties of miniature endplate potentials at normal, developing, degenerating, denervated and repressed nerve–muscle junctions

Agent	Junction				
	Normal	Developing	Degenerating	Denervated (Schwann cells)	Repressed
Nerve stimulation	e.p.p. or a.p.	No effect	Variable	No effect	No effect
Nerve section	Abolished	Abolished	—	No effect	Abolished
Frequency (min^{-1})	10–60	0.2–60	0.2 to very high, in bursts	0.2–10	0.2–10
La^{3+}	Increased frequency	Increased frequency	Increased frequency	No effect	No effect
K^+	Increased frequency	Increased frequency	Increased frequency	No effect	No effect
Hypertonic solutions	Increased frequency	Increased frequency	Not tested	No effect	Increased frequency

with La^{3+} ions or by raised K^+ in the bathing medium[10]. Unlike Schwann-released m.e.p.ps, they were very sensitive to increased tonicity of the bathing medium, and no 'silent period'[10,11] elapsed before they could be seen (we saw no Schwann m.e.p.ps in axolotl muscles kept fully denervated for up to 42 d, in spite of recording close to old endplates).

The unique properties of these m.e.p.ps (Table 1), together with their territorial distribution in experimental muscles (Fig. 2), and their dependence on the integrity of the posterior nerve, distinguish them from other classes of m.e.p.ps, and we suggest that they are released from nerve terminals whose normal function has been repressed (and possibly also from developing inappropriate nerve terminals whose maturation has been arrested).

On the basis of our observations we propose that repression of transmission at inappropriate junctions in axolotl muscle is due to a change in or loss of the Ca^{2+}-dependent mechanism[16,17] involved in transmitter release from nerve terminals. At normal and developing endplates La^{3+} ions seem to affect transmitter release by activating this Ca^{2+}-dependent process[12], and the effect of K^+ ions on transmitter release requires the presence of Ca^{2+} (ref. 14). On the other hand, the effect of hypertonic solutions is independent of the presence of Ca^{2+} (ref. 14). Also, the properties of the 'repressed' m.e.p.ps were mimicked by botulinising normal nerve terminals, and it has recently been shown that m.e.p.p. frequency in botulinised nerve–muscle preparations is returned to normal by treating them with a calcium ionophore in the presence of high external Ca^{2+} (ref. 18). We do not know how long functionally repressed junctions may remain physically present in a muscle.

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Are transmitter release statistics meaningful?

A BASIC premise of synaptic physiology is that neurotransmitter is released from motor nerve terminals in multimolecular packets called quanta[1,2]. The rate of release of quanta is low at rest, but when an action potential invades the nerve terminal there is a large increase in the mean rate of release leading to the generation of an endplate potential. It has been found that the distribution of the number of quanta released by an action potential is accurately described by a binomial distribution[3–8]. The assumption which underlies the binomial process is that n independent units (quanta) each have the same probability of success (release), p, on a single trial (stimulation). The parameters n and p can be estimated from the mean and variance of the binomially distributed variable. Although it is not clear what the physical correlates of n and p might be, it has been suggested that n may refer to the number of releasable quanta[1], or to the number of release sites which can be activated on stimulation[6]. The changes in the apparent values of n and p which occur during synaptic processes such as facilitation[6,8] and depression[7], have been interpreted as providing evidence for particular mechanisms which may underly those processes.

Recent studies have shown that the probability of quantal release, either spontaneous or evoked, is increased after a spontaneous release[10,11]. This suggests that in normal conditions p and/or n may vary with time. The bursts of spontaneous release produced by the addition of brown widow spider venom occur at localised areas of the nerve terminal[12], suggesting that spatial variations in p or n may exist. Studies of the changes in the apparent values of n and p which occur during neuromuscular depression have been interpreted as indicating that the release probability may be different for different quanta[7]. These experimental findings have led us to investigate the implications of a distribution of quantal release probabilities. As a result of these studies we believe that the application of the binomial distribution to transmitter release may not be appropriate.

A simple example can easily demonstrate the effects of a distributed probability of release. Consider an experiment in which the mean quantal content, m, is equal to 16 and the variance, σ_m^2, is equal to 8. Since $m = np$ and $\sigma_m^2 = np(1-p)$ for a binomial process it is easy to show that $p = 0.5$ and $n = 32$. An identical mean output and variance could, however, be obtained from a population of 50 quanta, 10 with a probability of 0.8 and the rest with a probability of 0.2 Simple examples like this show that a distributed probability can cause large errors in the estimation of binomial parameters from the mean and variance of the quantal contents. Before this conclusion can be accepted however, it is necessary to demonstrate that the shape of the distribution of quantal contents cannot be distinguished from the binomial prediction by goodness-of-fit statistics. A computer simulation was necessary to generate and compare the actual and the predicted distributions. A number of different probability distributions were investigated, produced by defining a new variable $z = \log_e p/(1-p)$ and generating a normal distribution of z with mean z and variance σ_z^2. The z values were then converted back into p values. The main advantage of this approach is that a family of distributions of p can be generated which have no quanta at $p = 0$ or $p = 1$. A trial was then run by generating rectangularly distributed random numbers and comparing them in turn with the elements of the p distribution. If the value of p for a quantum was more than the random number then that quantum was counted as being released in that trial. A number of trials were run until a smooth distribution of quantal contents was obtained. Figure 1 illustrates the results of one simulation in which most of the 100 quanta had very low probabilities of release. One hundred trials were simulated. There is no significant difference between the simulated distribution of quantal contents and that predicted for a binomial process ($\chi^2 = 4.98$ with 5 d.f., $P > 0.25$). It is interesting to note that only 1% of the quanta have probabilities of release equal to, or greater than the estimated mean probability of release.

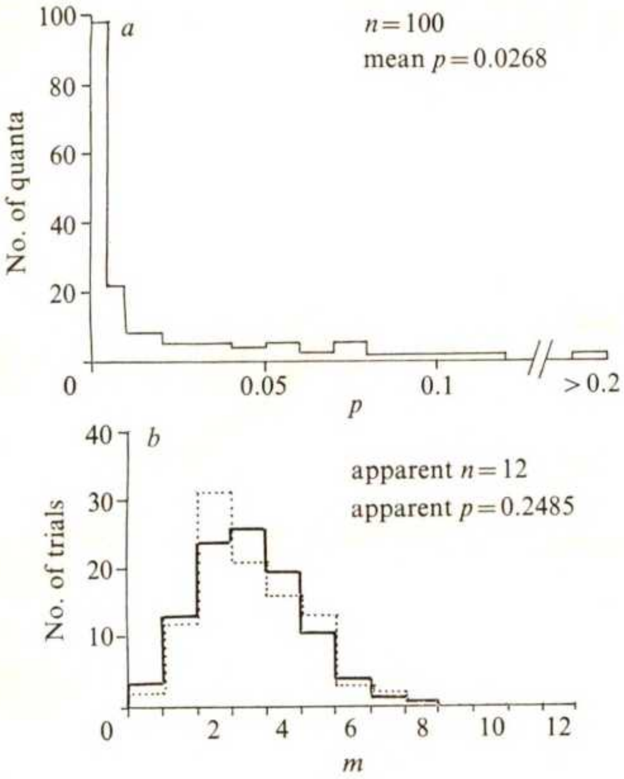

Fig. 1 The results of the simulation. *a*, Distribution of the probability of release for 100 quanta ($z = -5$, $\sigma_z^2 = 2$). *b*, Simulated distribution of quantal contents (- - -) and the binomial prediction (———) with parameters derived from the ean and variance of the simulated distribution.

Is it possible to establish that the quantal probabilities are not equal? In general, this is not possible for moderately sized data sets, but in special circumstances it should be possible to record quantal contents on single trials that exceed the estimated value of *n*. Such a result is strong evidence against the validity of binomial statistics, and has been observed at the crayfish neuromuscular junction[8].

The results are clear. Should the release probability for individual quanta be distributed about a mean value, instead of having no variance, then the application of binomial analysis is unjustified even if the considerable practical difficulties involved in the estimation of quantal content can be overcome. Thus, in order to assess the statistics of transmitter release, it is essential to estimate the distribution of the release probabilities. Without this information it is difficult to see how a meaningful analysis can be achieved.

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Replication of EBV in epithelial cells during infectious mononucleosis

INFECTIOUS mononucleosis is caused by primary infection with the Epstein–Barr virus (EBV)[1,2]. This virus is generally held to be trophic for B lymphocytes[3–6], but only latently infected cells expressing a nuclear antigen (EBNA)[7] have been found among circulating lymphocytes during infectious mononucleosis[8]. In spite of this, EBV is present in the oropharyngeal secretions of most patients with infectious mononucleosis, and virus continues to be shed in many normal persons for extended periods[9,10]. The source of this virus, a cell type which freely permits replication of EBV, has never been identified. An intriguing link has recently been advanced between EBV and the malignant epithelial cells of undifferentiated carcinoma of the nasopharynx. Patients with this malignancy maintain high titres of antibodies to the virus[11]. The tumour itself, which consists of malignant epithelial cells with many infiltrating lymphocytes, contains the EBV genome[12]. The EBV genetic material is in the malignant epithelial cells[13,14], however, and not in the infiltrating lymphocytes which are predominantly T cells. Although superinfection of nasopharyngeal carcinoma cells with EBV leads to the expression of specific early viral antigens[15], these cells, like B lymphocytes, are only latently infected *in vivo*. The primary cell type infected by EBV probably occupies a pivotal position in the pathogenesis of both infectious mononucleosis and nasopharyngeal carcinoma. We report here evidence directly from human material that EBV replicates in epithelial cells of the oropharynx.

Patients with infectious mononucleosis were selected on the basis of a compatible clinical syndrome, a positive 'Monospot test' (Ortho-Diagnostics, Inc.) and high antibody titres to EBV early antigen (EA) and EBV viral

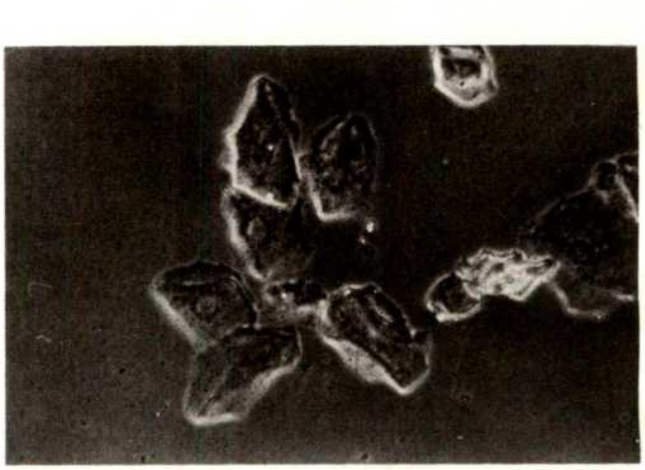

Fig. 1 cRNA–DNA *in situ* cytohybridisation of oropharyngeal epithelial cells from an EBV-antibody negative donor. 8×10^5 c.p.m. ^{3}H-cRNA (specific activity 1×10^7 c.p.m. μg^{-1}) was hybridised under coverslips as described[19,20] for 22 h at 66 °C. Slides were then washed four times in NaCl 0.3 M, sodium citrate 0.03 M (2X—SSC), treated with RNase 20 μg ml^{-1} for 1 h at 37 °C, washed again four times with 2X-SSC, dipped twice in 70% ethanol and then twice in 95% ethanol. Kodak Nuclear Tract NTB2 emulsion was used for autoradiography; exposure was for six weeks at −20 °C. In this specimen, which did not contain EBV, a few scattered grains are seen over each of several epithelial cells. (Unstained, phase contrast, × 475.)

capsid antigen (VCA) (ref. 16). Throat washings obtained by gargling with phosphate-buffered saline solution (PBS) were centrifuged at low speed; the supernatant fluids were passed through a 0.45-μm filter and stored at −70 °C in 10% foetal calf serum. Specimens were tested for EBV by an assay based on stimulation of DNA synthesis in human umbilical cord lymphocyte cultures as described previously[17]. Those throat washings which gave maximal stimulation of thymidine incorporation at 37 d of culture were selected for *in situ* cytohybridisation with EBV-specific complementary RNA (cRNA). Previous efforts to demonstrate EBV-infected cells in oropharyngeal material by indirect or anticomplement immunofluorescence techniques had failed due to intense nonspecific fluorescence.

Centrifugation of each throat washing yielded readily identifiable epithelial cells. The cell pellets were washed twice in PBS, resuspended in ice-chilled methanol–glacial acetic acid (3 : 1) for 5 min and fixed to glass slides for *in situ* cytohybridisation. EBV DNA was purified[18] and EBV-specific ^{3}H-labelled cRNA was synthesised and hybridised as described previously[19,20] to cell smears prepared from specimens known to contain EBV. Cells from a donor without serological evidence of previous EBV infection were also tested. After extensive washing and RNase treatment, hybridised cRNA was demonstrated by autoradiography[19].

Cells from the EBV-negative donor demonstrated a low background of grains diffusely scattered over numerous epithelial cells (Fig. 1). Specimens known to contain transforming virus presented an entirely different picture. The number of grains over almost all epithelial cells was higher than in the control preparations (Fig. 2*a*, *b*). Much more striking, however, was the appearance of dense accumulations of grains over a minority of epithelial cells, often seen in close proximity to other cells which hybridised far less of the EBV-specific cRNA (Fig. 2*a*). The density of grains was uniform over most of these EBV-containing cells, but occasionally there were denser accumulations of grains over the nucleus (Fig. 2*b*).

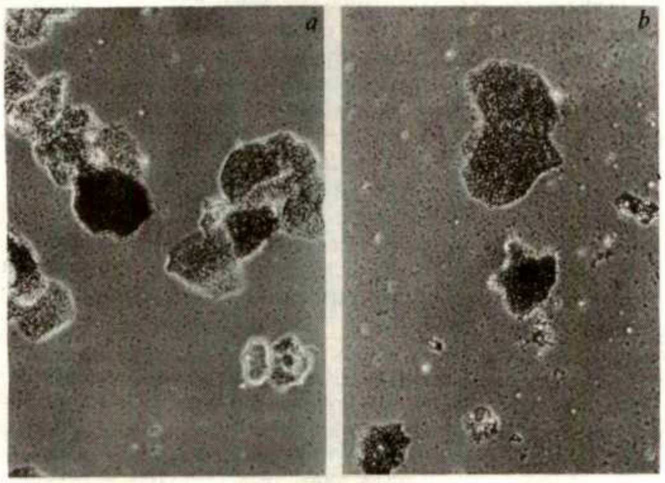

Fig. 2 *a*, Cytohybridisation of oropharyngeal epithelial cells from a patient with infectious mononucleosis and mild pharyngitis. Anti-EA was 1:160 and anti-VCA was 1:160; supernatant fluid from this throat washing resulted in a sevenfold stimulation of thymidine incorporation by cord lymphocytes after 37 d of culture. Note one cell over which there is a dense accumulation of grains while adjacent cells have hybridised relatively little EBV-specific cRNA. *b*, Cytohybridisation of oropharyngeal cells from a second patient with infectious mononucleosis and moderately severe tonsillopharyngitis. Anti-EA was 1:40 and anti-VCA was 1:160; supernatant fluid resulted in fivefold stimulation of cord lymphocytes. Several EBV-infected cells are present; in one, grains are concentrated over the cell nucleus. (*a*, *b*, Unstained, phase contrast, ×674.)

The number of grains over the most positive of these EBV-infected cells was equivalent to that seen over P3HR-1 cells that replicate EBV DNA and produce virus (Fig. 3). As the sensitivity of *in situ* cytohybridisation is rather low (about 60 EBV genomes per cell)[20], the results indicate a very high number of EBV genomes per positive epithelial cell. This itself constitutes strong evidence not only for infection with EBV but also for *in vivo* replication of the virus.

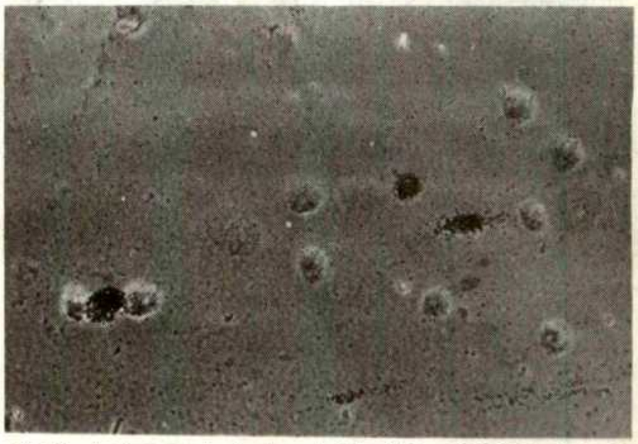

Fig. 3 A virus-producing lymphoid cell line (P3HR-1), derived from African Burkitt's lymphoma, which was hybridised at the same time as the oropharyngeal cells. Four EBV-producing cells can be seen, whereas other, latently infected lymphoid cells did not hybridise appreciable amounts of cRNA. The density of grains over the virus-producing cells is equivalent to that over EBV-infected epithelial cells in Fig. 2*a* and *b*. (Unstained, phase contrast, ×674.)

Since there is virus in the throat, virus might adsorb on to epithelial cells. This would give fewer grains, however, not localised on the nucleus and not spare adjacent cells. To control for this possibility, normal epithelial cells were exposed to a filtrate of a mononucleosis throat washing before cytohybridisation. This exposure produced little adsorption of virus as disclosed by hybridisation, even though the filtrate contained transforming virus and in spite of the presence of EBV-infected epithelial cells in the original mononucleosis specimen (Fig. 4).

When stained with methylene blue–basic fuchsin, the EBV-infected cells showed the small centrally placed nuclei and abundant cytoplasm typical of epithelial cells. There was no obvious cytopathic effect, but morphology following NaOH denaturation was less than optimally preserved.

Our results indicate that EBV infects and replicates within epithelial cells during the course of infectious mononucleosis. This observation answers several longstanding questions. It suggests that the epithelial cell is the source of the virus found in throat washings, consistent with the observation that virus shedding can occasionally be localised in the anterior oropharynx where lymphoid tissue is scant, but epithelium abundant, rather than the posterior pharynx and tonsillar areas[10]. Infection of the oropharyngeal epithelium may also account for the intense pharyngitis often seen in infectious mononucleosis. Virus presumably reaches the tonsils by way of the epithelium, where infection and transformation of the B lymphocyte occurs[21]. The transformed B lymphocyte then evokes a cytotoxic cellular response, characterised by the atypical lymphocyte which may by itself be largely responsible for the systemic manifestations of the disease[22,23].

It has been difficult to reconcile the presumed lymphotrophic nature of EBV with the presence of EBV DNA in the malignant epithelial cells of nasopharyngeal carcinoma. Although the lymphocyte has long been considered

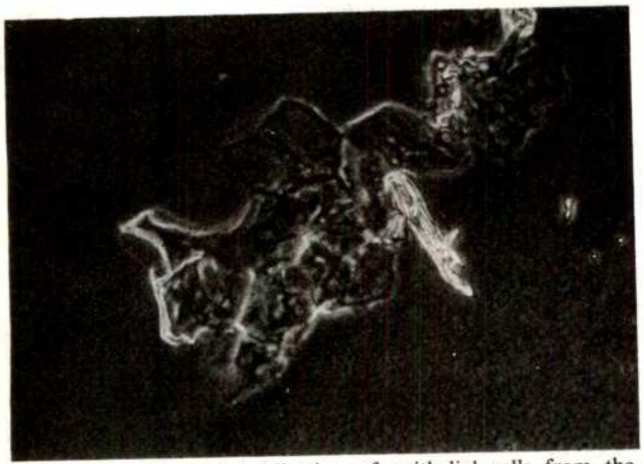

Fig. 4 *In situ* cytohybridisation of epithelial cells from the throat of a patient without antibodies to EBV after adsorption with supernatant fluid from a throat washing that contained transforming virus. Cells collected from the normal donor were washed twice in PBS and resuspended in the supernatant filtrate from the mononucleosis patient described in Fig. 2*a*. The normal cells were allowed to adsorb virus for 2.5 h at 4 °C, washed twice in PBS, fixed in methanol–acetic acid and processed for cytohybridisation as described in Fig. 1. There are only scattered grains over the cluster of epithelial cells. (Unstained phase contrast, ×674.)

the primary cell infected with EBV, it may well be that the primary cell is instead the epithelial cell. No other cell type has been shown to support EBV replication *in vivo*. Epithelial cell infection may be predominantly lytic and productive. Occasionally, however, perhaps influenced by genetic factors, there may be arrest of viral DNA replication, formation of EBV DNA episomes[24], and transformation of the epithelial cell. Such a transformed cell may be the progenitor of nasopharyngeal carcinoma.

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5′-Terminal nucleotide sequences of polio virus polyribosomal RNA and virion RNA are identical

POLIO virus is a positive strand virus; it has a mRNA of the same 5′ to 3′ polarity and size as its virion RNA (ref. 1). It also has a protein covalently linked to its 5′-terminal end forming a 5′-terminus of protein–pU–U–A–A–A–A–C–A–Gp (refs 2, 3). This contrasts with other viral RNAs that either have di- or triphosphate 5′-termini (negative strand RNAs)[4–6] or $m^7G^{5'}ppp^{5'}N(m)p$ . . . termini (most positive strand RNAs)[7,8]. The 5′-linked protein on poliovirion RNA is also found on at least two-thirds and perhaps all of the nascent chains of the replicative intermediate isolated from infected cells[3]. This suggests that the protein is linked to the RNA at a very early stage of replication, perhaps as a primer during the initiation of RNA synthesis[2,3]. The 5′-terminus of polio virus polyribosomal RNA lacks the protein and instead terminates in pUp . . . (refs 9, 10). Thus, it is possible that the protein initially present on the newly synthesised RNA chains is cleaved from that fraction of the RNA molecules destined to become mRNA. To determine the nucleotide sequence relationship between the polio virus RNAs containing and lacking the protein, we have isolated and sequenced the 5′-terminus of poliovirus polyribosomal RNA. We show here that its nucleotide sequence is identical to that of the 5′-terminus of virion RNA.

To identify the 5′-terminal oligonucleotide of poliovirus polyribosomal RNA we used the characteristic that distinguishes it from all other oligonucleotides in the RNA, the presence of a pUp residue. The total mixture of RNase T_1 oligonucleotides was fractionated by ionophoresis on cellulose acetate in the first dimension and homochromatography on polyethyleneimine (PEI)–cellulose plates in the second dimension[11,12] (Fig. 1*a*). To find the 5′-oligonucleotide, regions of the fingerprint were scraped from the PEI–cellulose plate and digested with RNases T_1, T_2 and A. The products were separated by paper electrophoresis at *p*H 3.5 (ref. 11). Only one spot on the fingerprint gave rise to pUp which was identified by its characteristic mobility just slower than orthophosphate[3]. An arrow indicates the pUp-containing spot in Fig. 1*a*. The rest of the regions analysed gave rise only to 3′-monophosphates. The identification of the pUp-containing oligonucleotide as the 5′-terminus of polyribosomal RNA is supported by its absence from the fingerprint of virion RNA (Fig. 1*b*, arrow). For virion RNA, the protein-linked 5′-terminal T_1 oligonucleotide would have been excluded from the second dimension because of its slow mobility in the first dimension[3].

The intensity of the pUp-containing spot was weaker than that of the neighbouring spots suggesting that it either was present in a submolar amount or that it contained fewer phosphates than the adjacent oligonucleotides. The two oligonucleotides to the right of the pUp-containing spot were determined to be about 18 and 20 nucleotides long. This was based on a quantitation of the RNase P_1 digestion products relative to the radioactivity in the

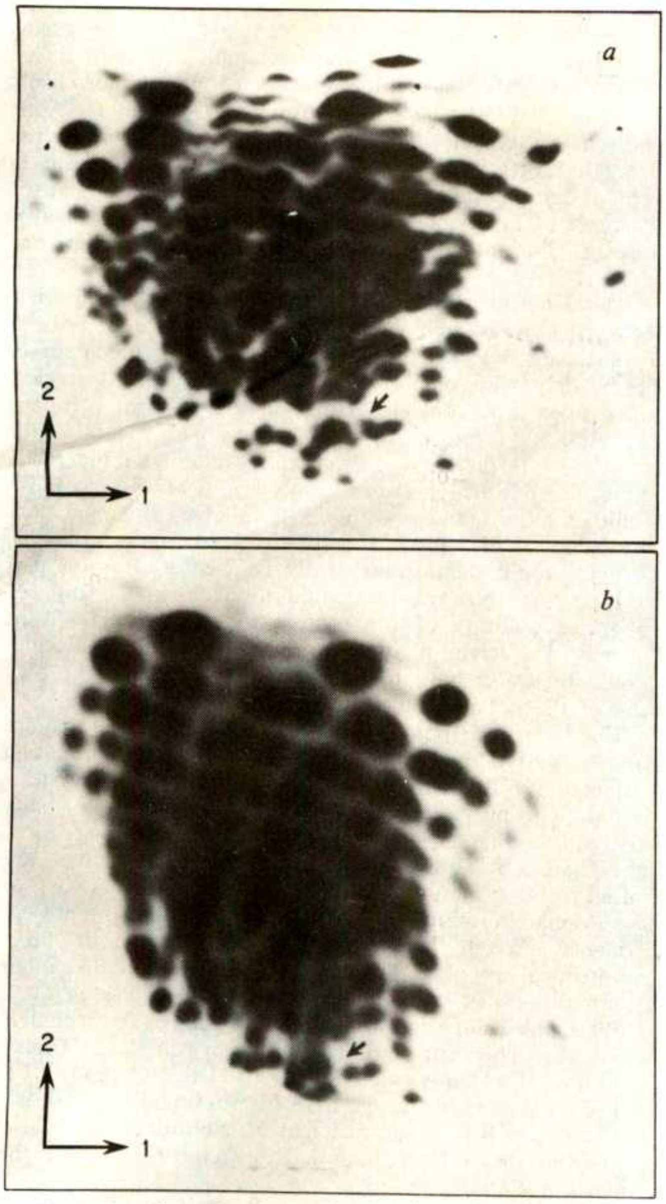

Fig. 1 Two-dimensional separation by electrophoresis and thin-layer chromatography of RNase T_1 oligonucleotides derived from polio virus polyribosomal and virion RNA. Polio virus-specific RNA labelled with ^{32}P-orthophosphate (New England Nuclear) was prepared by infecting actinomycin D-treated HeLa cells in suspension with polio virus type 1 as described previously[3,9]. RNA from purified virions labelled with ^{32}P (collected 6 h post-infection) was prepared by extraction with sodium dodecyl sulphate–phenol (SDS–phenol) and purified by sucrose gradient centrifugation as described previously[17]. Polio virus polyribosomal RNA was prepared from cells collected 3.5 h post-infection as described previously[9,16]. In summary, a cytoplasmic extract was taken and the polyribosomes collected on a sucrose cushion[17]. The polyribosomal RNA was precipitated with 2 M LiCl in the presence of 0.5% SDS at −20 °C. The precipitate was extracted three times with phenol–chloroform–isoamyl alcohol (25:24:1) and the RNA was recovered by ethanol precipitation and centrifugation. The RNA was further purified by oligo(dT) cellulose chromatography and sucrose gradient centrifugation. The peak fractions of 35S RNA were pooled and the RNA wax precipitated by two volumes of ethanol. Yields of between 1 and 3×10^7 c.p.m. (Cherenkov radiation) of ^{32}P-labelled polyribosomal RNA were regularly obtained from 4×10^8 cells and 60 mCi ^{32}P-orthophosphate. Purified polyribosomal and virion RNAs were digested with RNase T_1 for 30 min at 37 °C in 3 µl of 10 mM Tris, *p*H 7.4, and 1 mM EDTA containing 10 µg of RNA and 200 U ml^{-1} of T_1 (Calbiochem). The resulting oligonucleotides were subjected in the first dimension to electrophoresis on Cellogel strips (Chemeton, Milan) at 2,500 V for 70 min at *p*H 3.5 (ref. 11). Separation in the second dimension was by chromatography on plastic-backed polyethyleneimine–cellulose thin-layer plates (20×20 cm, Brinkmann Instruments)[11,12]. Homomixture C (ref. 11) digested for 2 min with 1 M KOH was used for the second dimension. *a*, Autoradiogram of a fingerprint of polio virus polyribosomal RNA; *b*, autoradiogram of polio virus virion RNA. In (*a*) the arrow indicates the position of the pUp-containing 5′-terminal oligonucleotide and in (*b*) the arrow indicates the absence of the spot from the virion RNA. Different batches of homomixture were used in (*a*) and (*b*).

5′-GMP. Correction for the unequal labelling of the four monophosphates was done as described previously[3] and in the legend to Table 1. As shown below the length of the 5′-terminal oligonucleotide is only 9 nucleotides (10 phosphates). Apparently the additional 5′-phosphate in the terminus substantially retards the mobility of the oligonucleotide during homochromatography.

Table 1 Ribonuclease A and U_2 products derived from the 5′-terminal oligonucleotide of polio virus polyribosomal RNA

	Product	Yield*
RNase A†:	A–A–A–A–Cp	1.0
	A–Gp	1.0
	pUp	0.7
	Up!‡	0.9
RNase U_2§:	[pUp, Up, Cp, $(Ap)_n$]	
	[pUp, Up, $(Ap)_n$]	
	A–Ap	
	C–Ap	
	Gp	
	Ap	

*The yield of RNase A products was calculated relative to A_4C. Corrections for unequal labelling of the four nucleoside monophosphates were done[3]. The ratios of the radioactivity of the 5′ monophosphates to that of the 3′ monophosphates were found to be 1.0 for CMP, 0.8 for AMP, 0.6 for GMP and 1.6 for UMP. In the case of Gp, where the nearest neighbour was unknown, a specific activity of 1.0 was used for the phosphate.

†Digestion conditions were: 30 min at 37 °C in 5 µl of 20 mM Tris, *p*H 7.5, and 2 mM EDTA, containing 15 µg of yeast RNA (Sigma) and 7.5 µg of RNase A (Worthington).

‡All the uridine monophosphate was recovered as 2′,3′ cyclic UMP.

§Only the composition of the two largest RNase U_2 products is indicated because exact sequences were not determined. The U_2 digestion conditions were: 2 h at 37 °C in 10 µl of 15 mM sodium acetate, *p*H 4.5, and 1 mM EDTA containing 0.01 U of U_2 (Calbiochem). In these conditions the U_2 products presumably contained 2′,3′ cyclic monophosphate termini.

Further purification of the oligonucleotide was required for sequencing purposes and was accomplished by elution of the oligonucleotide and fractionation on a two-dimensional polyacrylamide gel[6,13]. A major component migrated in the first dimension together with the bromophenol blue dye and in the second dimension slightly faster than it (Fig. 2). A few faint spots representing contaminating oligonucleotides from the first two-dimensional fingerprint could also be observed. Only the major spot contained pUp and therefore must have been the 5′-terminal T_1 oligonucleotide. Molar yields of secondary digestion products (Table 1) established the purity of this oligonucleotide.

To determine the sequence of the oligonucleotide, it was eluted, digested with RNase A or RNase U_2, and the products were separated by ionophoresis on DEAE-paper at *p*H 3.5 (ref. 11). RNase A digestion gave rise to four products, which were identified as A–A–A–A–Cp, A–Gp, pUp, and Up! on the basis of their migration relative to marker oligonucleotides and subsequent base composition analysis. All four products were present in approximately equimolar amounts (Table 1). The order of the RNase A products was established from the RNase U_2 digestion products (Table 1). The product C–Ap showed that the RNase A products A–A–A–A–Cp and A–Gp must have been linked in that order. The Up residue therefore must have been between pUp and A–A–A–A–Cp and we conclude that the sequence of the total oligonucleotide is pU–U–A–A–A–A–C–A–Gp. The sequence of the two large RNase U_2 products must have been pU–U–A–A–A–A–C–Ap and pU–U–(A_n)–Ap considering the RNase A digestion products. These products are consistent with the known specificity of RNase U_2 (ref. 11) and confirm the sequence. Thus, the nucleotide sequences of the 5′-terminal T_1 oligonucleotides from polio virus virion and polyribosomal RNA are identical, but the polyribosomal RNA lacks the protein at its 5′-end. We have also analysed the complete RNase T_2 products derived from the oligonucleotide on a two-dimensional thin-layer chromatography system[14] and found no evidence for any modified bases in the oligonucleotide (data not shown).

We have described a method for purifying to homogeneity the 5′-end T_1 oligonucleotide from polio virus polyribosomal RNA. Although the oligonucleotide contains only nine bases, its 5′-phosphate causes it to migrate slowly during homochromatography through PEI–cellulose positioning it in the region of the fingerprint where it is detectable as an individual spot. To purify it sufficiently for sequencing, further fractionation was needed and the two-dimensional electrophoretic system of De Wachter and Fiers[13] provided the needed resolution. This system fractionates mainly on the basis of base composition and length and would be expected to show less effect from the 5′-phosphate.

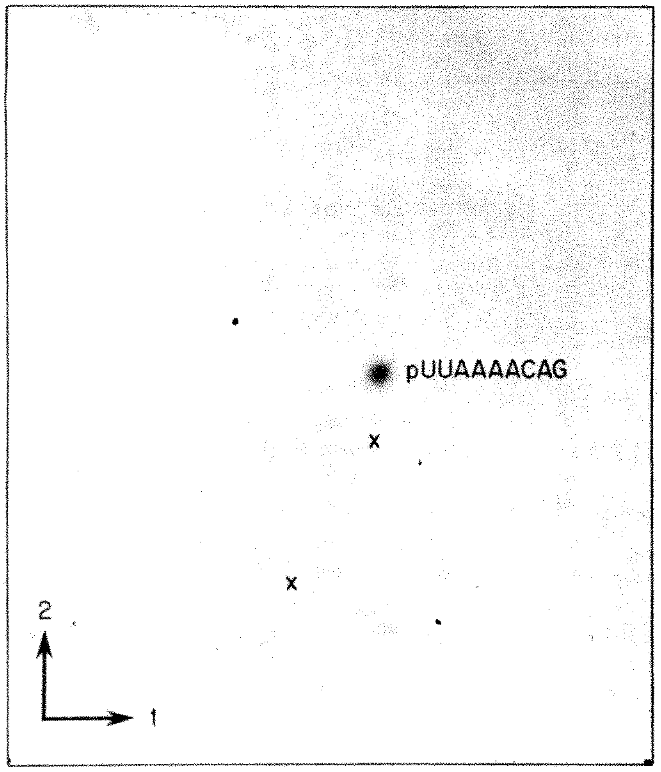

Fig. 2 Purification of the 5′-terminal oligonucleotide of polio virus polyribosomal RNA by two-dimensional gel electrophoresis. The pUp-containing T_1 oligonucleotide from the PEI–cellulose plate (Fig. 1*a*) was eluted with triethanolamine bicarbonate and fractionated by two-dimensional polyacrylamide gel electrophoresis as described previously[6,13]. The first dimension (10% acrylamide, 6 M urea, *p*H 3.5) was run until the bromophenol blue dye had migrated 20 cm. The second dimension (21.8% acrylamide in Tris-citrate buffer, *p*H 8.0) was run until the bromophenol blue dye had migrated 8 cm. The upper × indicates the position of the bromophenol blue dye and the lower × that of xylene cyanol. The sequence of the 5′-terminal oligonucleotide is as indicated.

The replicative intermediate isolated from polio virus-infected cells contains as an average about six growing strands[15]. Quantitation of the protein–pUp in these chains indicated that at least two-thirds contain a protein linked to their 5′-end[3]. Since mRNA is infectious[3], it most likely contains all of the genetic information in virion RNA. It is therefore unlikely that the polyribosomal RNA is derived from a larger precursor molecule by any mechanism in which one or more 5′-terminal nucleotides are lost by cleavage. This conclusion is supported by the identity of the 5′-terminal nucleotide sequences in virion and mRNA.

These considerations suggest that initiation of polio virus RNA synthesis must occur by one of three possible mechanisms: (1) the 5′-terminal protein or a precursor of it, possibly with one or several covalently-linked nucleotides (e.g. protein-pU_{OH}), could act as a primer in the initiation of synthesis of all strands. Subsequently, the protein would be cleaved from that fraction of the RNA molecules destined to become mRNA. (2) RNA synthesis could initiate *de novo* with UTP, followed by rapid condensation of the protein with all RNA molecules. The protein would then subsequently be cleaved from a part of them. (3) RNA synthesis could initiate as in (2), followed by rapid addition of the protein to only a part of the strands. The triphosphate end on the rest of the molecules would then also have to be modified to generate the pUp end of the polyribosomal RNA.

The latter two possibilities seem unlikely. They require that a pyrimidine triphosphate should be the initiating nucleotide. But, virtually all known RNA synthetic systems initiate with purine triphosphates[16]. Furthermore, possibility (3) requires that an enzyme cleave off the two 5′-terminal phosphates; in other systems where triphosphates are initiators the mature molecule retains either a di- or triphosphate terminus. Therefore we believe that the present evidence strongly favours possibility (1) but until direct biochemical proof of the model is provided the other alternatives must be considered possible.

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reviews

Botanical dimension in Darwin's thinking

R. H. Richens

Darwin and His Flowers: The Key to Natural Selection. By Mea Allan. Pp. 318. (Faber and Faber: London, 1977.) £6.95.

It remains obscure why evolution became scientifically respectable just when it did. Heritable variation at the varietal level was well known and accepted by nurserymen, animal breeders and biologists. The notion of macroevolutionary change was also in the air, as purveyed by Lamarck, Chambers and Charles Darwin's grandfather Erasmus, although less scientifically respectable. Malthus' essay had been widely read. It was not even the case that a vast body of cumulative evidence had to be assembled to vindicate the notion of macroevolution, and it seems that Wallace only required three days to excogitate the theory that Darwin laboured over for so many years.

Perhaps what had been lacking till 1859 was a protagonist in the right club, namely the small group of biologists accepted by themselves and by the general public as representing the forefront of validated scientific thinking. Neither Erasmus Darwin, Chambers nor Wallace were in this club. Charles Darwin, in spite of his lack of formal training, was.

From the point of view of the history of science, then, it becomes more relevant to enquire what led Darwin to sponsor evolution than to ask at what stage the evidence pointed that way. Miss Allan now provides us with some new perspectives. In the course of a very well illustrated account of Darwin's botanical activities, she shows how greatly Darwin was taken up with plants, even though his formal botanical qualifications were thin.

It is clear that Darwin was greatly moved by his encounter with equatorial forest. Possibly, no botanist is most perceptive in his native environment. It is notorious how botany still retains clear evidence of its temperate origins and how potently a small dose of the tropics contributes to theoretical advancement. On the other hand, one can suppose that familiarity with tropical vegetation can be as soporific to its denizens as familiarity with temperate vegetation is to its. It is also clear from Miss Allan's book that plants attracted Darwin as research material, not only as exhibiting the operation of natural selection, but as doing so without the theoretical complication of "willed" activity.

Miss Allan presents her material in roughly chronological order: Darwin's childhood, Edinburgh and Cambridge, the voyage of the Beagle, the *Origin of Species,* his researches on the fertilisation of orchids, climbing plants, plant variation under domestication, insectivorous plants, cross- and self-fertilisation, and heterostyly. Darwin's work is presented very largely in his own terms and in sufficient detail to illustrate his remarkably acute power of observation and experimental ingenuity.

The chief value of Miss Allan's book, beside the pleasure of just looking at it, is the re-orientation that it suggests for the interpretation of Darwin's thought. The botanical dimension has certainly been undervalued in the past. In detail, however, Miss Allan cannot be our guide. Neither her geology—coprolites as fossil excreta—nor her evolutionary and genetic theory—largely presented in the semi-Lamarckian, pre-Mendelian categories of Darwin's later writings—has been brought up to date.

When it comes to personal interpretation, Darwin becomes unreal; the man has become a scientific hero figure with the inevitable distortions that this produces. Her remarks on Darwin's "illness" are trivial. But one imagines that Miss Allan makes no pretensions in these areas. What she has done, and done well, is to present a vivid picture of the role of plants in Darwin's scientific life, and it was Darwin himself who wrote that "a traveller should be a Botanist, for in all views plants form the chief embellishment". ☐

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Continuing Newtonian disputes

The Correspondence of Isaac Newton. Volume 6: 1713–17–18. Edited by Rupert A. Hall and Laura Tilling. Pp. xxxviii+499. (Cambridge University: Cambridge and London, 1976. Published for the Royal Society.) £25.

The core of this sixth volume of the Newton correspondence is made up of a collection of letters and documents relating to the controversy between Newton and Leibniz over the calculus, the commencement of which was noticed in the previous volume. Only a few of these letters and documents are from Newton himself, although some of these are of great interest, such as the hitherto unpublished No. 1053*a.* On the British side the most common correspondent is Newton's indefatigible champion John Keill, and on the continental side it is Leibniz himself and Johann Bernouilli, with the Italian Conti—eventually a proselyte to the Newtonian camp—acting as intermediary. The editors speculate how Newton might have spent his time if he had not been involved in this controversy with Leibniz. They might equally have speculated how differently things could have turned out if the controversy had never arisen in the first place, or at any rate had not become envenomed up to the point that British mathematicians effectively cut themselves off for more than a century from the main stream of continental mathematics. It was a strange nemesis that Newton, who by his work in optics, and in the physical, astronomical and philosophical aspects of the *Principia,* had made so vast a contribution both to scientific progress and scientific attitudes in the eighteenth century, and had raised the prestige of British science so high, should have exerted through his mathematical attitudes and techniques so retrograde an influence on British mathematics and mathematical physics in the same century.

This volume witnesses the *dénouement* of the longstanding Newton/Flamsteed controversy in which Flamsteed finally had the better of Newton, largely it seems as a result of the eclipse of certain of Newton's supporters after the accession of George I in 1715. Flamsteed, who now had friends at court, greeted this change of power with glee (letter No. 1151) and rightly anticipated that it would transform his relations with Newton and the Royal Society over the question of the contentious *Historia Coelestis*. In the event Newton and the referees were forced to call off their campaign against Flamsteed to whom the remaining copies of the *Historia Coelestis* were returned—mostly for burning—together with the manuscripts of the observations on which the work itself had been based, and which Flamsteed then used to prepare his own version of the *Historia Coelestis,* published by his wife some years after his death.

A surprising feature is the absence of any letters between Newton and Clarke relating to the latter's famous controversy with Leibniz although this fell entirely within the span of the present volume. As the editors suggest, this could simply have resulted from Clarke's intimacy with Newton and the fact that they both lived in London, although it should not be assumed that Newton played the same sort of role in the Clarke/Leibniz controversy, as in the controversy over the calculus.

Cotes' letter of 22 December 1713 to Newton (No. 1029) marks the dismal end to the magnificent correspondence relating to the preparation and publication of the second edition of the *Principia.* Whatever the reason, resentment at Newton's failure to thank him for his vast unpaid labours on the *Principia,* or increasing ill health—he was to die young in June 1716—Cotes coldly rejected all the corrigenda and addenda suggested by Newton in a previous letter. As for some 20 additional errata listed by Newton in the same letter, Cotes had noticed some of them for himself, but had foreborn to enter them in his own list of errata lest he should appear "too diligent in trifles". He ends his letter with a final implicit reproof by pointing out that he had himself silently corrected some hundreds of errors without troubling to acquaint Newton of their existence.

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The standard of editing maintains the high level of previous volumes and there is an excellent introduction including a very helpful survey of the controversy over the calculus. An unfortunate omission, however, is any reference in the page opposite the list of contents to the late J. F. Scott's editorship of Volume 4 of the Newton correspondence. Nor do all the footnotes relating to mathematical questions have that luminous clarity which one is entitled to expect. This I found particularly true of note 1 to Newton's notes on Leibniz's 'Tentamen' (letter 1069*a*). This work of Leibniz certainly provides good justification for Voltaire's quip that "Leibniz is come to confuse the sciences" but unfortunately the footnote itself compounds this confusion. Thus it is misleading to say that the theory of Leibniz is "congruent mathematically with that of Newton in the *Principia*" unless by congruent is meant that Newton and Leibniz arrived by entirely different mathematical methods at the same inverse square result for the radial force for motion in an ellipse. Nor is it entirely true that the difference between the two treatments "lies in the *physical* interpretations of Newton and Leibniz". For although it is true that Leibniz posits a miraculous *ad hoc* 'harmonic' vortex to account for the circulatory motion of a planet in conformity with Kepler's second law, nevertheless a careful examination shows that this vortex plays no part whatsoever in the actual mathematical argument in which the one physical factor is the assumption that the force directed towards the focus will be measured—for a given time interval—by the deviation between the actual curved path and the inertial path along the tangent or approximating cord. It is extraordinary that Leibniz should have hit on this notion —one of the most profund physical insights in the *Principia*—before reading the *Principia* itself.

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Illuminating mental darkness

Light and Life. By Lars Olof Björn. (Hodder and Stoughton: London, Sydney, Auckland and Toronto, 1976.) Hardback £3.45; paperback £1.75.

As its title implies, this small book seeks to cover a vast field. All situations where light interacts with living organisms provide a subject for discussion in this book. It begins very naturally with photosynthesis and then moves into the animal kingdom for such topics as bioluminescence and vision. Photoperiodism and spatial orientation with respect to light, influence both animals and plants. There remain some essentially anthopocentric subjects—namely, suntan, artificial illumination and photochemical smog.

The book is based on the original Swedish edition, but some of the later chapters are new additions. It has been well translated and reads very freely. The level is about that of *Scientific American* and, like that journal, it relies heavily on simplified diagrams for the communication of information. Technical detail and jargon are avoided and the emphasis is essentially on readability. This, coupled with its accurate, full and up-to-date content, make it the sort of book one can pick up and read cover to cover. It will appeal most especially to sixth-formers and first-year undergraduates but should, and undoubtedly will, also be read by more advanced students and researchers.

When a small book attempts to cover so much it is not difficult to find gaps. A surprising one is the potential of solar energy for space and water heating and energy farming. One could also criticise the inevitable shallowness of parts of the text. It is not a work of reference to which one might resort for a detailed exposition of, say, photorespiration in C−3 plants or phototaxis in *Euglena.* But at least such topics are mentioned and the basis of their interest and importance is adequately conveyed. The provision of a carefully selected further reading list amply covers the needs of the student whose interest has been fired and who needs to delve deeper. Like the Sun god, Aton, to whom this book is dedicated, Professor Björn should succeed in illuminating the mental darkness of many.

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Computer image processing

Digital Image Restoration. By H. C. Andrews and B. R. Hunt. Pp. xviii+238.) Prentice-Hall International: Englewood Cliffs, New Jersey, and Hemel-Hempstead, UK, 1977.) £19.95; $31.75. *Digital Picture Analysis.* Edited by A. Rosenfeld. Pp. xiii+351. (Springer: Berlin and New York, 1976.) DM72; $29.60. *Image Science Mathematics.* Edited by C. O. Wilde and E. Barrett. (Western Periodicals: North Hollywood, California, 1976.) $37.50.

THESE three volumes—a textbook, a set of essays ranging from high energy physics to cytology, and a collection of contributions to a highly specialised symposium—give a vivid idea of what is happening in computer image processing and of the directions that the subject is likely to take in the near future, although the very new tse computers (D. H. Schaeffer and J. P. Strong, *Proc. IEEE*, **65**, 129–138, 1977) are not discussed.

The textbook, by Andrews and Hunt, is an extremely useful account of the present state of digital image processing, from a mathematical standpoint; it is very much more than a revised edition of Andrews' earlier text on a similar subject (*Computer Techniques in Image Processing*, Academic: New York and London, 1970), for the material has developed and the points of emphasis changed so rapidly that a completely new text has been written. It is clear and carefully organised; the numerous pitfalls and still unsolved problems are signposted; the notation is easily readable; and in regions where tempers have tended to fray in the past, the authors remain commendably objective, their assessment of digital against optical restoration being a good example. The book is divided into three parts: an introduction, covering image formation, recording and models; two chapters on degradations and determination of parameters characterising these; and four chapters on restoration, covering noniterative methods that use Fourier space, the numerous filters generated by a linear algebraic approach and finally, non-linear algebraic methods. *Digital Image Restoration* has only one direct rival: *Digital Picture Processing* by Rosenfeld and Kak (Academic: New York and London, 1976; for review, see *Nature*, **264**, 142, 1976). Both are excellent and the overlap is less than it might seem from a superficial comparison, for the authors' interests are rather different even though much common ground is covered.

Digital Picture Analysis contains five long contributions: automatic, remote sensor image processing by Haralick; radiographic image analysis by Harlow and Dwyer; image processing in high energy physics by McIlwain; digital picture analysis in cytology by Preston; and picture analysis in character recognition by Ullmann. In contrast to the text by Andrews and Hunt, these essays show clearly the successes and problems of processing real large two-dimensional arrays in very different fields. Pattern recognition, in particular texture analysis, is as important here as image restoration; and inevitably very similar problems occur throughout the various applications. It was an excellent idea to bring this material from such different branches of applied science together in a single volume as the subject is still sufficiently young for extensive cross-fertilisation to be taking place. The essays are of a high standard and are written to be comprehensible to a non-specialist audience.

Finally, we turn to the contributions to the symposium on image science mathematics, held in California in November, 1976. The tone here is very different from that of the other two books under review, for the reader is expected to be conversant with "the story so far", and now ready to read on. There are papers on non-linear restoration, in particular by Frieden on the maximum entropy technique; there are new results on the Karhunen–Loève transform by Jain; Reed *et al.* pursue their work on transforms over finite fields; Kreznar explores the consequences of the lack of a two-dimensional fundamental theorem of algebra; Hunt surveys the (severe) problems of non-linear restoration; Keng and Fu discuss a syntax-directed method of analysing Landsat images; and there are many others. In short, these Proceedings, offset from authors' typescripts, contain much exciting and up-to-date material but they are not for the novice; and furthermore, fuller accounts of the same work are now appearing in the appropriate journals.

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Comparative immunology

Comparative Immunology. Edited by J. J. Marchalonis. (Blackwell Scientific: Oxford and London, 1976.) £15.50.

THE past 15 years have seen a surge of interest in developmental immunobiology. *Comparative Immunology* provides a timely review of the extensive list of research papers in this field and gives insight into phylogenetic and ontogenetic studies currently in progress in the authors' laboratories. Contributions from more than 20 expert immunologists fit together well to make this a really first-rate, readily-readable review.

As a preface to the chapters on phylogeny of immunity, Nossal outlines the cellular, molecular and genetic basis of the complex immune system found in mammals, and Rosser, drawing attention to evolutionary principles, suggests that lower organisms may well have evolved different forms of defence. This suggestion is, in part, borne out by the chapters that follow, on the evolution of cellular immunity. Thus although Cooper presents evidence that all invertebrates, even the Protozoa, can distinguish self from non-self, and postulates the existence of primordial cell-mediated immunity in annelids and echinoderms, Jenkin concludes that recognition by invertebrate phagocytes is based on a system of serum proteins which, although analogous in function, differ in structure from the immunoglobulins that are unique to the vertebrates. Insight into the physicochemical and biological properties of non-mammalian immunoglobulins can be gained from later chapters.

Ontogenetic aspects of cellular and humoral immunity in amphibians, reptiles, birds and mammals are examined in some detail. Rowlands describes immune responses of prototherian and metatherian mammals and discusses how these studies are relevant to the study of immune development. Du Pasquier's elegant work reveals that amphibians are proving invaluable in elucidating the mechanism of tolerance, the origin of antibody diversity, the role of the thymus and the nature of the T-cell receptor. Furthermore, Cohen illustrates that "Linnaeus' loathsome amphibians" possess an unusual array of immunological phenomena, while Balls and Ruben make central use of these poikilotherms in excellent reviews on the phylogeny of neoplasia and immune reactions to tumours, and on the nature of cell co-operation.

The book should prove invaluable to all immunobiologists wishing to learn in depth about the evolution of immunity in the entire animal kingdom and of the advantages that can be gained by using simple model systems to study central issues in immunology.

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Water and aqueons solutions

Liquids and Solutions: Structures and Dynamics. By P. Kruus. Pp. 592. (Marcel Dekker: New York and Basel, 1977.) SFrs. 145.

THIS book is based on a graduate course and seminars, and is intended for physical chemists and people with related interests—for example, engineers and biologists. Certain topics are deliberately omitted—for example, glasses, melts, quantum liquids, liquid metals, and liquid crystals. A feature is the considerable attention given to water and aqueous solutions. These topics are of the greatest interest to other scientists but are often neglected because water (or an aqueous solution) is extremely difficult to handle theoretically. The author does not try to deal with any topic in depth but has produced what amounts to a very interesting series of review articles with a very large number of appropriate references. These will certainly be valuable both to specialist research workers, and to workers in related fields, for many years to come. One is struck both by the author's vast reading, and by his ability to summarise a particular field and put it into proper relation with other fields.

The book is divided into three parts. Part A deals with theoretical approaches: what are the forces between molecules or ions and how do we allow for their effects? Model treatments, distribution function theory and the theory of electrolytes are all summarised with appropriate references, but the reader is always left wanting more. Two chapters on transport properties and molecular motions can only be described as sketchy.

Part B is concerned with experimental studies. We can distinguish between what we can deduce from the equilibrium properties and transport coefficients (chapters 8 and 9), what we can deduce by bombarding the liquid with neutrons or by imparting various forms of energy to it (chapters 10 and 11, and 14–16), and what we can learn by using a typical molecule as a probe in magnetic resonance and spectroscopic studies (chapters 12 and 13). Each chapter has some specific examples of the application of each method. A considerable amount of very interesting material is given, but one would have welcomed a further chapter explaining exactly what sort of information can legitimately be expected from each method. For example, it may seem elementary to the author that because of wavelength considerations, ultrasonic and light-scattering studies tell us nothing about the distribution function but a great deal about critical fluctuations; but such information would be welcome to some of his likely readers.

Part C gives very brief accounts of thermodynamics statistical mechanics, electromagnetics and quantum mechanics. This is the weakest feature of the book. Although over 70 pages are used, these chapters are no substitute for a proper textbook, as the author himself recognises. If these topics are to be treated at all, why not include hydrodynamics also? I would like to have seen these pages used instead in expanding the excellent parts A and B, some chapters of which have quite clearly had to be unduly compressed.

Despite this tactical error, I adhere to my opinion that the author has done a good job in summarising what is now a considerable body of knowledge and that the result will be helpful to research workers. The emphasis on water and aqueous solutions is particularly to be commended at the present time, much of the material given being hard to find elsewhere.

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Complexities of intelligence

Intelligence: Nature, Determinants and Consequences. By E. B. Brody and N. Brody. Pp. x+241. (Academic: New York and London, 1977.) $12.50; £8.85.

INTELLIGENCE TESTING has been with us for some seventy years, and the results used—and sometimes misused—for important social and educational purposes during much of this period. From the start, at least two irreconcilable lines of thought about intelligence test scores can be traced. The first was at the outset peculiarly British, initiated by Galton and developed by Spearman, suggesting that there exists a central, inherited general intellectual capacity, fixed and unalterable, and directly measurable by tests. The second, which arose from the work of Binet in Paris, stressed the potentially plastic nature of intellectual functions.

Today, the questions of plasticity of intelligence, the degree of its heritability both within and between groups and races, remain central and controversial. The debate has been sharpened by Jensen's article (1969) which suggested a strong inherited component in the well-attested average negro–white IQ differences, and by revelations in 1974 by Jensen and by others, that some of the major post-War data of Sir Cyril Burt, the arch-hereditarian, were worthless.

The book by Brody and Brody addresses itself to problems such as these, sketching initially a little of the historical background, considering the work of Cattell and Guilford on the structure of the intellect, and outlining the quantitative characteristics of global indices. The relationship between test scores and achievement is reviewed and the respective roles of genetic, biological and social environmental factors as determinants are considered. A critical chapter is devoted to the problems of racial differences, birth order and family size, and the volume concludes with a review of the use of tests in the educational system, in selection and in clinical assessment.

Like many new American books in this field, this volume reflects the radical change which has taken place in recent thinking on what intelligence tests measure, their social and educational applications, and the constellation of variables which affect their results. It is up-to-date, clearly written and indicative of an unusual width and depth of knowledge. Surprisingly, however, it is weak in the section on stability and change in individual intelligence during the lifespan, considering in detail only one major longitudinal study; indeed, it underestimates the amount of relative change occurring in children reared in fairly constant environments. It also pays too little attention to the many careful studies on larger IQ changes following major environmental shifts. Yet the authors are rightly doubtful of "critical period" hypotheses which suggest that only early interventions may be rewarding for the disadvantaged. They fail, however, to spell out in detail the implications for successful interventions, in particular duration and totality.

Here and there, one can fault the authors' statements: for example, the results of the important Dutch Famine Study are open to alternative explanations than the one they favour, and they do not reveal any awareness of this controversy. The omission of an author index and the sketchiness of the subject index are also surprising. All in all, however, this book achieves its aim—a balanced account of the major issues. It will be valuable to those who seek an introduction to understanding these complexities.

A. D. B. Clarke

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Recent scientific and technical books

Mathematics

BENTON, Jr., Stanley H. The Hamiltonian-Jacobi Equation: a Global Approach (Mathematics in Science and Engineering: a Series of Monographs and Textbooks, Vol. 131.) Pp.xi+147. ISBN-0-12089350-9. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) $13.50; £9.60.

BINMORE, K. G. Mathematical Analysis: a Straightforward Approach. Pp.x+257. ISBN-0-521-21480-7. (Cambridge, London and New York: Cambridge University Press, 1977.) Hardcover £9.75; Paperback £3.75.

BLYTH, T. S. Module Theory: an Approach to Linear Algebra. Pp.400. ISBN-0-19-853162-1. (Oxford: Clarendon Press; London: Oxford University Press, 1977.) £9.50 net.

BRIDGE, Janes. Beginning Model Theory: The Completeness Theorem and Some Consequences. (Oxford Logic Guides.) Pp.vii+143. ISBN-0-19-853157-5. (Oxford: Clarendon Press; London: Oxford University Press, 1977.) £4.50 net.

DREYFUS, Stuart E., and **LAW**, Averill M. The Art and Theory of Dynamic Programming. (Mathematics in Science and Engineering: a Series of Monographs and Textbooks, Vol. 130.) Pp.xv+284. ISBN-0-12-221860-4. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) $18.50; £13.15.

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obituary

Pehr Edman

Dr Pehr Edman, FRS, died on 19 March 1977, in Munich, at the age of 60. In an eventful and distinguished scientific career on three continents, he was undeniably one of the most influential figures in advancing our understanding of the chemical structure of proteins.

Pehr Edman was born in Stockholm on 14 April 1916. Though his undergraduate training was in medicine, very early on he was attracted to the field of biological chemistry, then in its infancy, and in the 1940s carried out (with U. S. Von Euler and E. Jorpes) some of the earliest work on the chemical characterisation of angiotensin. He later spent a year at the Rockefeller Institute in the laboratory of John H. Northrop, studying the effects of tyrosinase on several proteolytic enzymes.

In retrospect it is difficult to envisage the primitive state of methods for peptide analysis at that time. An amino acid analysis of a protein was a major undertaking; sequence analysis of even a small peptide was a chemical *tour de force*. Pehr Edman clearly recognised the need for beter analytical techniques for proteins, and was to devote almost his whole scientific career to developing them.

He approached this task with thoroughness and attention to detail illuminated by a clarity of thought characteristic of the man. His critical insight was to see that earlier attempts at an end group procedure for peptides by Max Bergmann and Emil Abderhalden could be converted into a sequential degradation procedure by simply substituting sulphur for oxygen in the reagent used. This led to one of the most important, (and most often cited) papers in modern biological literature: 'A Method for the Determination of the Amino Acid Sequence in Peptides' published in *Acta Chemica Scandinavica* in 1950.

From this point Edman's international reputation was established, and he could easily have taken the method as it then was and applied it to structural studies on proteins of biological interest. High honours would have surely and rapidly followed, as they did to others. (Sanger, earlier, and Moore, Stein, Anfinsen and Edelman, later, received Nobel Prizes for their studies on protein structure and function). Pehr Edman typically took a more difficult road. He set out to perfect the sequential degradation method, a task that was to occupy the rest of his life. The way he went about this is in itself a fascinating story. Suffice it to say that in 1967, with a talented colleague in Melbourne, G. Begg, he published in the first issue of the *European Journal of Biochemistry* an article describing an instrument, the 'sequenator,' for the automatic determination of sequences in proteins. This instrument is now produced commercially and is in use in many laboratories around the world. Its availability has vastly accelerated progress in molecular biology. For this work Pehr Edman was awarded in 1971 the Commonwealth Brittania Award, and the rare Berzelius Gold Medal (the eighth awarded this century). In 1974 he was elected to the Royal Society of London.

At a personal level Pehr Edman was an interesting and complex man. Close or intimate relationships were rarely established even by those who worked in the same field or who followed his methods closely. I was privileged to be one of a small number of his students and close associates. These included John Sjoquist, Birger and Margareta Blombäck, Agnes Henschen (later his wife), Frank Morgan, and of course his colleague for fifteen years, Geoffrey Begg. Pehr rarely spoke of personal matters, but it seems that the end of his first marriage in divorce and some disappointments in local recognition and advancement in Sweden may have contributed to his decision to move to Melbourne in 1957 as Director of St Vincent's Hospital School of Medical Research.

Initially this must have been more a self-imposed exile than a positive career decision. The laboratories at St Vincent's prior to his arrival were nonexistent. Apart from the late Professor John Hayden and one or two others, the staff at St Vincent's in 1957 had little idea of the real potential of Edman's work or of his outstanding scientific abilities. As his work had no obvious immediate clinical relevance he was largely ignored by the hospital. Pehr Edman's reserved, not to say withdrawn, personality contributed to this. The end result was that he worked for many years in virtual isolation both from St Vincent's Hospital and with a few exceptions from the Australian scientific community. He gained at least the local reputation of being unsociable and difficult. On the contrary, he was, I believe, a rather shy person who simply did not have the sort of extroverted nature required to make his presence felt in the scientific wilderness of Australia in which he found himself.

To his close associates and friends, and to scientific visitors to his laboratory he was a totally different man—warm, helpful and unfailingly courteous. However, he was uncompromising in his attitude to anything he regarded as scientifically unsound. In his own work he was overwhelmingly competent. He enjoyed working at the bench, designed and made his own instruments and was an excellent glassblower. In the European tradition, the laboratory was run on disciplined lines. No short-cuts or shoddy techniques were tolerated. He put great emphasis on self-reliance and careful use of equipment. At least in the early days in Australia he refused to buy the commercially produced micropipettes of Lang–Levy design. Each was made and calibrated in the laboratory and kept with scrupulous care. Chemicals were never used straight out of the bottle; before each experiment began he would personally carry out a careful redistillation or recrystallisation of the required reagents. His approach was totally professional.

Pehr Edman was a man of cultivation, widely read, interested in music and the arts, and a gourmet cook. At morning and afternoon tea breaks at work his ritual was to sit down, light one of his favourite thin cigars and slowly drink a cup of very strong freshly ground coffee. The conversation, usually unrelated to science, reflected his wide interests and cosmopolitan attitudes.

Though at first he must have felt the scientific and personal isolation in Australia, Pehr grew to love his country of adoption and later became an Australian citizen. He particularly enjoyed fishing and bird-watching, and few scientific visitors from overseas escaped a day-long excursion to Sherbrooke Forest, near Melbourne, to catch a glimpse of the elusive Australian lyre bird.

In 1972 he left Australia for Munich to be head of a new protein chemistry unit at the Max-Planck-Institut für Biochemie at Martinsried. Unhappily he did not long survive in this new venture. He leaves a wife, Dr Agnes

Henschen-Edman, and two young children, Carl and Helena.

Though his career was tragically cut short, he succeeded brilliantly in the task he set himself. One wonders what scientific problem would not have succumbed to Pehr Edman's creative and single-minded endeavours.

H. D. Niall

C. J. Smithells

Dr Colin J. Smithells who died on 25 March 1977, held a variety of posts in the metallurgical field since he graduated in chemistry at Leeds University in 1914.

Immediately after graduation he joined the Army in which he served with distinction throughout the 1914–18 war reaching the rank of Major in the Gloster Regiment, gaining the Military Cross and being mentioned in dispatches.

In 1919 he joined the research staff of the General Electric Company at the new laboratories being set up by Sir Clifford Patterson at Wembley. There he was primarily responsible for the production and fabrication (by the new metallurgical techniques such as powder metallurgy) of the less well-known metals—with particular reference to tungsten and the manufacture of lamp filaments, receiving valves etc. His book *Tungsten* continues to hold the field and other books, such as *Gases in Metals* have established with authority the existing state of knowledge at the time they were written.

He was awarded the degree of DSc. by Leeds University in 1921. While still at GEC in 1937 he developed a Heavy Alloy (of tungsten nickel and copper) particulars of which were first published in *Nature* in 1937.

In 1938 he left the GEC to become General Manager of Lodge Plugs of Rugby where he was concerned with the use of platinum alloys until 1944 when he was appointed Director of Research of the British Aluminium Company. Here he was responsible for the setting up of the Company's new and now well-known Research Laboratories at Chalfont Park in Buckinghamshire.

In 1956 he retired from Chalfont Park to become Managing Director of Magnesium Elekton Limited, which post he held until he finally retired in 1963.

Throughout his working life he devoted time generously to the work of organisations which aimed to benefit the industry and profession of metallurgy. He was, in 1945, a Founder Fellow of the Institution of Metallurgists, of which he was President in 1951–52. In the following year he was President of the Institute of Metals and throughout gave much time to the work of government committees and industrial bodies such as the British Non-Ferrous Metals Research Association and the Aluminium Development Association. In 1949 he edited the first issue of the *Metals Reference Book*, a comprehensive collection of data which he saw through several new editions.

He was outstanding in the profession for his charm of manner and clarity of thought and speech and will be a great loss. He is survived by his wife Mary, two sons and three daughters.

G. L. Bailey

R. N. Singh

Professor R. N. Singh, formerly Head of the Botany Department of Banaras Hindu University, died from heart failure on March 9, 1977. He was internationally recognised as an authority on phycology, to which he contributed much original observation and in which he initiated new lines of research. Especially, he was distinguished for his work on nitrogen fixation by blue-green algae in rice fields, making contributions both to botanical knowledge and practical agriculture. Besides isolating and characterising various nitrogen-fixing species, he was one of the first to study the paddy field as an ecosystem. His use of the simple expedient of enclosing areas of alkaline "Usar" lands, so that ponds form in the rainy season and prolific growth of blue-green algae is encouraged, is a method of reclamation the full potential of which has not yet been realised. This and other work is described in his book, *Role of blue-green algae in nitrogen economy of Indian agriculture* (1961). He also carried out research on the taxonomy, morphology, physiology, radiation biology, genetics and virology of blue-green algae. In the course of this work he produced many mutant strains of blue-green algae, which have interesting implications for our understanding of morphogenesis in these organisms.

R. N. Singh was born on August 2, 1915, in the Deoria district of Uttar Pradesh and was educated at the U.P. College, Varanasi and Even Christian College, Allahabad. He obtained the M.Sc. degree in 1938 at the Banaras Hindu University while working under Professor Y. Bharadwaja. In 1946 he was awarded the D.Sc. of the same University. He worked in several famous laboratories abroad including the Department of Botany, University College London, the Department of Biochemistry, University of Wisconsin (where he participated in the preparation of the first cell-free extracts active in nitrogen fixation), and the Plant Research Laboratory, Michigan State University. He travelled widely and was a familiar figure at international conferences on phycological and limnological topics.

Besides being a creative research worker, Professor Singh was an outstanding teacher of plant physiology as well as phycology and many of his students now hold responsible posts in different parts of the world. He established a well equipped phycological laboratory in the Banaras Hindu University where he trained more than thirty Ph.Ds in various branches of phycology. After retirement he occupied himself with writing on recent developments in the study of blue-green algae. It is to be hoped that these nearly completed works will be published in due course. His sudden death is a sad loss to Indian science and phycology generally. He will be remembered with affection and gratitude by his many friends and students.

P. K. Singh
G. E. Fogg

Eli Lilly

Eli Lilly, former President and Chairman of Eli Lilly and Company of Indianapolis died on 24 January at the age of 91. He was a very remarkable man. From the age of 10 he worked with his father Josiah K. Lilly Senior, in the family business and in 1907 qualified as a pharmaceutical chemist from the Philadelphia College of Pharmacy and Science, where his father also had studied. Thereafter he developed an interest, ahead of his time, in efficiency of production methods, becoming superintendent of the manufacturing division in 1909, general superintendent in 1915, and vice-president from 1920 until 1932 when he succeeded his father as president. He guided the company through the difficult Depression years of the early 1930s and despite the economic downturn no employees were laid off. Under his overall guidance the production of insulin for diabetes was developed in the 1920s, the production of liver extract for pernicious anaemia, and of barbiturates, in the 1930s; and after the war production of penicillins and other antibiotics including erythromycin.

Lilly was a great philathropist. He endowed a memorial laboratory in 1936 at the Philadelphia College of Pharmacy and founded Lilly Endowment, Inc. in 1937 which over the years has given some 250 million dollars to charitable causes. His wide-ranging interests included educational and civic activities, stock-breeding and archaeology. His wife died in 1973 and their daughter in 1970. There are no immediate survivors.

Frank Hartley

announcements

On the Move

S. Sampanthar, Department of Mathematics, University of Salford, to Department of Mathematics, University of Calabar P.M.B. 1115 Calabar, Nigeria on 11 July 1977.

Details of changes of department, sabbaticals, where leave will be taken and so on should be sent to On the Move; there is no charge for this service.

Awards

Charles Earp of Standard Telecommunications Laboratories is the first recipient of the new award, the J. J. Thompson Medal, of the Institution of Electrical Engineers, for his work on communication and radio navigational aids.

W. E. Klinner was the first recipient of the Douglas Bomford Trust Prize from the Institution of Agricultural Engineers for the best technical paper, entitled 'The Field Treatment of Grass for Conservation', presented at the 1976 Annual Conference.

The European Physical Society has awarded the 1977 Hewlett–Packard Europhysics Prize (20,000 S.Fr.) to Professor Walter Eric Spear of the Carnegie Laboratory of Physics at the University of Dundee in Scotland for his work on a special form of semiconductor which is non-crystalline and can be prepared as thin films.

B. Heezen is the recipient of the 1977 Walter H. Bucher Medal for original contributions to the knowledge of the Earth's crust.

P. Richards received the 16th James B. Macelware Award for significant contributions to geophysical sciences.

Appointments

D. A. L. Saunders to the Chair of Architecture at the University of Adelaide, South Australia.

D. J. Cove to Professor of Genetics and Head of Department at Leeds University from 1 January 1978.

Dr R. S. Barnes, to Principal of Queen Elizabeth College from 1 April 1978.

Sir Richard Doll, OBE, Regius Professor of Medicine at the University of Oxford to chairman of the Committee of Management of the Institute of Cancer Research from 1 January 1978.

Professor F. L. Mastaglia, to the Chair of Experimental Neurology, University of Newcastle-upon-Tyne.

Dr M. J. Sewell, to a Personal Professorship in Mathematics at Reading University from 1 June 1977.

Dr R. F. Curtis, to Director of Agricultural Research Council Food Research Institute at Norwich on 1 October 1977.

D. Eccleston, to the Chair of Psychological Medicine from 1 October 1977, and also to Head of the Department of Psychological Medicine for five years from 1 October 1977.

Person to Person

Furnished 3/4-bedroom house, Manchester area, available 1 March 1978 to 28 February 1979. Contact M. Sampanthar, 25 Vaudrey Drive, Cheadle Hulme, Cheshire, UK.

Exchange furnished 5-bedroom house in Gainesville, Florida for similar convenient to Mill Hill, London, for 1 year, September 1977 to August 1978. Rental of London house also considered. Contact D. Duckworth, Box J-266, JHM Health Center, Gainesville, Florida 32610.

Exchange furnished, 7-room house south-east London, end August 1977 to July 1978, for house or apartment in Boston/Cambridge, Massachusetts area. Contact J. A. Patterson, Apt. C511, 77 Pond Avenue, Brookline, Massachusetts 02146.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

International Commission for protection against enviromental mutagens and carcinogens

This commission (ICPEMC) has been established to primarily approach the problems involved in minimising hazards of the increasing variety of mutagenic chemicals to which human populations are exposed. Current knowledge is to be reviewed with the aim of formulating recommendations to serve as bases for regulatory action.

Lady Tata Memorial Trust

The Trustees of the Lady Tata Memorial Trust for research in leukaemia have made the following International Awards for the Academic Year 1977–78. R. de N. Bastos (Spain), L. Gauci (Great Britain), P. Mignatti (Italy) (2nd year awards); H. Jacobs (Great Britain), ¼. Ravid (Israel) (New awards); E. Cardoso (Portugal), G. Grosslerner (France) (New awards from April 1977).

Meetings

25–29 July, **International Conference on Solar Building Technology,** London, UK (The Secretariat, International Solar Building Conference, North East London Polytechnic, Forest Road, London, UK).

15–17 August, **Symposium on Prospecting in Areas of Glaciated Terrain,** Helsinki (The Institution of Mining and Metallurgy, 44 Portland Place, London, UK).

2–5 August, **Cryogenic Engineering/Materials Conference,** Boulder, Colorado (Bureau of Conferences and Institutes, Academy 217, 970 Aurora Avenue, University of Colorado at Boulder, Boulder, Colorado 80302, USA).

23–26 August, **5th International Conference on Chemical Thermodynamics,** Ronneby, Sweden (Fifth International Conference on Chemical Thermodynamics, c/o RESO Congress Service, S-105 24 Stockholm, Sweden).

24 August, **19th Canadian High Polymer Forum,** Ottawa (Secretary–Treasurer, 19th Canadian High Polymer Forum, National Research Council of Canada, Division of Chemistry, Montreal Road, Ottawa, Ontario, K1A 0R9, Canada).

28–30 August, **Satellite Symposium to the VI International Meeting of the International Society for Neurochemistry: Endorphins,** Brescia, Italy (F. G. Lorenzini, Via Monte Napoleone, 23, 20121 Milan, Italy).

31 August–2 September, **2nd Drag Reduction Conference,** Cambridge, UK (Organising Secretary, 2nd Drag Reduction Conference, BHRA Fluid Engineering, Cranfield, Bedford, UK).

12–16 September, **Symposium on Unconventional Photographic Systems,** Oxford, UK (Royal Photographic Society of Great Britain, 14 South Audley Street, London W1, UK).

14–16 September, **The Oil Industry and Microbial Ecosystems,** Warwick (The Institute of Petroleum, 61 New Cavendish Street, London W1, UK).

Reports and Publications Other countries—May

Smithsonian Contributions to Zoology, No 247: The Entocytherid Ostracods of North Carolina. By Horton H. Hobbs, Jr., and Daniel J. Peters. Pp. iv + 73. (Washington, DC: Smithsonian Instutition Press, 1977. For sale by US Government Printing Office.) [45

Bulletin of the Florida State Museum, Biological Sciences. Vol. 20, No. 4: Fossil Sirenians and Desmostylids from Florida and Elsewhere. By Roy H. Reinhart. Pp. 187–300 $3.85. Vol. 21, No. 1: Variation and Relationships of Some Hispaniolan Frogs (Leptodactylidae, *Eleutherodactylus*) of the *Ricordi* Group. By Albert Schwartz. Pp. 1–46. $1.70. Vol. 21, No. 2: The Natural History of the Alabama Map Turtle, *Graptemys pulchra* Baur, in Alabama. By Robert M. Shealy. Pp. 47–111. $2.10. (Gainesville, Elorida: Florida State Museum, University of Florida, 1976.) [45

United States Department of the Interior: Geological Survey. Bulletin 1418: Weak-Field Magnetic Susceptibility Anisotropy and Its Dynamic Measurement. By William F. Hanna. Pp. iv + 73. (Washington, DC: US Government Printing Office, 1977.) [55

Food and Agriculture Organization of the United Nations. Food and Environment: Reconciling the Demands of Agriculture with Global Conservation. Pp. 44. (Rome: FAO; London: HMSO, 1976.) £1.62. [55

Université du Québec. Un Réseau de Personnes—Ressources à Votre Service. Pp. 164. (Québec: Université du Québec, Institut National de la Recherche Scientifique, 1977). [55

Organisation for Economic Co-operation and Development. Energy Production and Environment. Pp. 107. (Paris: OECD; London: HMSO, 1977.) F.22; £2.60; $5.50. [65

OECD Planning Group on Science and Technology for Developing Countries. Report of the Steering Group on Pest Control Under the Conditions of Small Farmer Food Crop Production in Developing Countries Pp. 83. (Paris: OECD, 1977.) [65

Battelle Memorial Institute. The President's Report and Annual Review 1976. Pp. 28. (Columbus, Ohio: Battelle Memorial Institute, 505 King Avenue, 19/7.) [95

Université du Qubéec. L'Institut National de la Recherche Scientifique—Descriptive Brochure, Pp. 78. (Québec: Institut National de la Recherche Scientifique, 1977.) [95

World Health Organization. Technical Report Series, No. 604: Wholesomeness of Irradiated Food—Report of a Joint FAO/IAEA/WHO expert Committee. Pp. 44. Sw.fr.6; $2.40. The Primary Health Worker: Working Guide, Guidelines for Training, Guidelines for Adaption. Experimental edition. Pp. iv + 337. Sw.fr.20; $8. (Geneva: WHO; London: HMSO, 1977.) [95

Fondation Universitaire. 56^{e} Rapport Annuel, 1975/1976. Pp. 59. (Bruxelles: Fondation Universitaire, Rue d'Egmont, 11, 1977.) [95

United States Department of the Interior: Geological Survey. Professional Paper 440-KK: Data of Geochemistry. Chapter KK: Compilation of Stable Isotope Fractionation Factors of Geochemical Interest. Sixth edition. By Irving Friedman and James R. O'Neil. Pp. vi + 12 + figures 1–49. Professional Paper 939: Artificial Recharge to a Freshwater-Sensitive Brackish-Water Sand Aquifer, Norfolk, Virginia. By Donald L. Brown and Willian D. Silvey. Pp. v + 53. (Washington, DC: US Government Printing Office, 1977.) [95

Energy, Mines and Resources Canada. Geological Survey of Canada. Paper 76–7: Geological Survey of Canada Radiocarbon Dates XVI. By J. A. Lowdon and W. Blake, Jr. Pp. 21. $3. Etude 76–19: Le Quaternaire Recent de la Region du Cours Inferieur de la Grande Riviere, Quebec. Par Jean-Serge Vincent. Pp. 20. Paper 76-28: Publications on the Geology of the Arctic Islands (District of Franklin) by the Geological Survey of Canada. Compiled by R. L. Christie. Revised July 1976. Pp. 37. Paper 76-30: Inertial Navigation for Flight Path Recovery. By R. L. Grasty, J. F. R. Gower and B. M. Oliver. Pp. 12. $3. Paper 76-35: Upper Cretaceous and Tertiary Coal-Bearing Strata in the Drumheller-Ardley Region, Red Deer River Valley, Alberta. By D. W. Gibson. Pp. 31 + 4 plates. $4.20. Paper 77-6: The Geosciences in Canada-1976, a Status Report. Edited by C. R. Barnes. Prepared by the Canadian Geoscience Council. Pp. 75. gratis. (Ottawa: Geological Survey of Canada, 1976 and 1977.) [105

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nature

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Peking exacts too high a price

THE cathedral city of Durham in the north of England is to be the site of an unusual battle in a week's time. For the question of the representation of the People's Republic of China in an international scientific union is to be put to the test, and, as in the international political bodies, the precondition of membership is to be the expulsion of Taiwan. There is by no means universal agreement that this is a worthy action.

Last year the International Union of the Geological Sciences (IUGS), meeting in Sydney, Australia, voted that the Academia Sinica of Taipei was not a valid body to be affiliated to IUGS, and went on to accept the Academia Sinica of Peking's application for membership. The Australian government had made its own small contribution to this decision by refusing visas to the Taiwanese delegates. The International Council of Scientific Unions (ICSU) didn't think much of all this and told IUGS to look into ways in which Taiwan could be reinstated.

The next union in line for a conditional application by the People's Republic of China was the International Union of Geodesy and Geophysics (IUGG), and its secretary-general, Professor P. Melchior of Brussels, is convening a council meeting, followed by an extraordinary general assembly (for the purposes of ratification) to coincide with a routine scientific gathering in Durham. Professor Melchior, who knows the People's Republic of China well, has a statement by the IUGG Bureau to fortify him. It is generally expected that this statement will assert that Taiwan is a province of the People's Republic, and so only Peking should represent China, and Taiwan's representation should cease.

Very few delegates would question the need for the People's Republic to be absorbed into international bodies, but it is to be hoped that many will baulk at the price. Gone, clearly, are the days when Taiwan could claim to represent mainland China, but equally, the day is still far off when Peking can claim *de facto* to be able to represent Taiwan, which indisputably runs and finances its own independent scientific activities. If Rhodesia can be a full member of IUGG alongside the United Kingdom, has the People's Republic, not yet even a member, strong grounds to dictate that the academy of an irritant government, running a quite respectable programme in geophysics, should be wiped off the international scene? Certainly such actions don't seem to match up in any way to the grand talk of the universality of science and its freedom from politics.

Many countries have already filed postal votes without bothering to hear the debate in Durham. Others are coming with instructions to admit the People's Republic, probably for the reason that they would rather be friendly with Peking than Taipei. So it is generally expected that Peking will have its way. Against all this the United Kingdom is to raise the question of universality and various constitutional issues —the latter a little unwillingly, because IUGG's constitution was not fashioned with such squabbles in mind and so could simply give legalistic nit-pickers a field day. The United States is expected to follow the same tack and will warn that back home the government won't like this sell-out of Taiwan by scientists and will look rather carefully and with due deliberation at the annual subscription that IUGG levies.

If the vote goes Peking's way, ICSU must, in logic, take at least as firm a line as it did with the geologists—firmer, preferably, because IUGG cannot claim to be ignorant of ICSU's views. And IUGG will have to face up to a problem in 1979 when it plans to hold a large meeting in Melbourne, Australia. ICSU rules now state that if any host country refuses visas to *bona fide* scientists wishing to attend a meeting, the relevant union must put pressure on that country. The pressure would presumably be in the form of a threat to move the meeting elsewhere or even cancel it. On the past form, Australia will turn away scientists from Taiwan. (IUGG may expel Taiwan, but it still recognises the right of qualified scientists from 'every part of the world' to participate as individuals in its meetings.) So IUGG must soon make its mind up not just over Taiwan's membership but also on some longer term consequences.

"If the international unions didn't exist, they wouldn't need to be invented", said one scientist last week who has had substantial experience with them. Even though they clearly serve a valuable purpose in helping a few scientists to travel and participate in international endeavours, the politicking that the People's Republic of China is trying to import could well seriously diminish what value the unions do possess. ☐

Cancer prevention: a question of priorities

Ernst L. Wynder of the American Health Foundation argues for the virtues of preventive medicine in combating cancer

RECENTLY, two independent studies have considered the relative contribution of environmental factors to the incidence of cancer*. Their common conclusion was that cancer is not an inevitable consequence of living, and that most cancers appear to be related to life-style factors. Estimates on the contribution of lifestyle to cancer incidence generally parallel each other (see below). The unanswered question is how we should react to our failure to curb those environmental factors that cause the continued high risk for many cancers.

As long ago as 1950, epidemiologic evidence established the causative association between cigarette smoking and lung cancer. Twenty-seven years later, tobacco usage still accounts for more than one-third of all male cancer deaths in the United States, in Britain and in other countries. Little did we expect that man's illusion of immortality would foster public apathy toward health messages related to smoking; nor did we foresee the unwillingness and inability of the medical establishment to assist significantly the motivation of the public to modify health behaviour. The opposition of commercial and agricultural interest, of course, was to be expected.

With the impact of these countervailing forces now so recognisable, the measure of success of a prevention-oriented approach to health maintenance seems to be inversely proportional to the responsibility one must assume for one's behaviour. It is thus far easier to launch a campaign directed against occupational and iatrogenic cancers than it is to infringe upon personal behaviour since, with the former, one can marshal the energy of government, the media and unions. It is no wonder that Doll regards the efforts necessary to solve the problem of nutrition-related cancers as an even more difficult task.

The identification and subsequent reduction of environmentally induced cancers requires a multidisciplinary undertaking, ranging from molecular biology to public health. Just as Comprehensive Clinical Cancer Centres can more effectively prescribe treatment regimens for cancer patients, a multi-faceted approach to environmental cancers would provide a vehicle for researchers to work in tandem on issues related to environmental cancers. The creation of Interdisciplinary Centres for Cancer Prevention is now called for.

The cross-fertilisation between epidemiologists, biologists, chemists and public health professionals has already proved constructive in focusing, directing and applying research efforts. For these efforts to succeed, however, the broad support of our academic and government institutions, and especially of the scientific community, is required.

Endeavours in the field of prevention are currently receiving only a fraction of available government or private funding, with the possible exception of investigations on industrial carcinogens. Economic considerations may force a change. As disease care costs continue to rise, prevention will necessarily attract greater attention to avoid bankrupting the existing medical care delivery system.

It has been said that those who do not learn from history are condemned to relive it. The lessons to be learned from scurvy, smallpox and cholera can be repeated for most cancers, as well as for other chronic conditions. Eradication of a disease is not dependent upon fully comprehending the disease mechanism. This applies to cancer prevention as well.

The question remains whether we will accept these lessons from history, particularly when personal and societal 'sacrifices' are demanded. A patient dying from a self-inflicted cancer would, in retrospect, certainly answer with an unqualified "yes." But what of the rest of us looking toward the future? The development and support of Interdisciplinary Centres for Cancer Prevention could help us reach the proper perspective on the values of preventive medicine. It could also draw us closer to the day when what we know to be avoidable disease will no longer plague the health and economic fibres of ours and future generations. □

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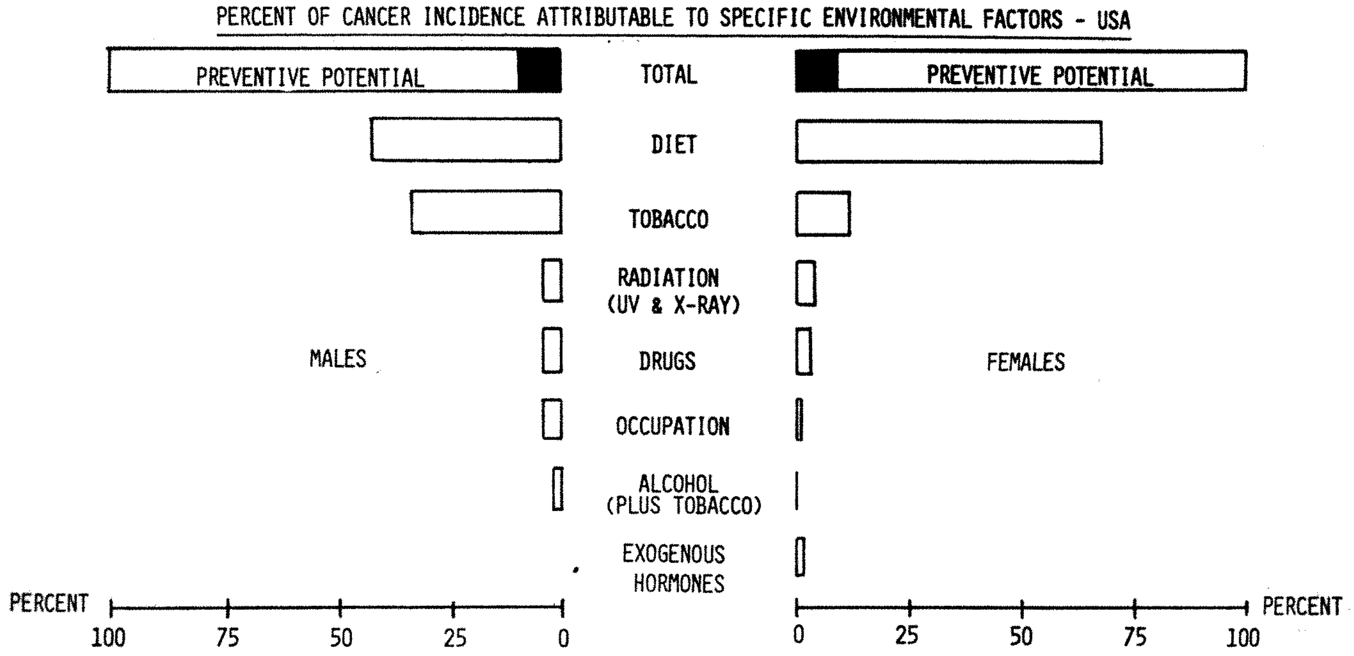
* Doll, R., *Nature* **265**, 589-596; Wynder, E. L. and Gori, G. B., *J. Natn. Cancer Inst.* **58**, 825-832.



Soviet genetics: new controversy

Zhores Medvedev reviews the development of human genetics in the Soviet Union since Lysenko

T. D. LYSENKO dominated Soviet genetics and biology for more than 25 years. In 1965, when his domination was over, genuine science was quickly restored to research and education. It was generally assumed that his political and ideological interference in the nature of genetic problems had been completely wrong and harmful. The notions of a 'Soviet' and a 'bourgeois' natural science had been almost forgotten. In spite of Lysenko's long 'dictatorship', there were still quite a few prominent, enthusiastic geneticists of the older generation who could build up research again, write new textbooks for schools and universities, and support and teach the younger generation.

For human genetics, however, the position was much more bleak. The subject was declared racialist and was completely destroyed in 1936–38. Most of its prominent representatives were arrested, and not one of them was found alive when Khruschev dismantled the GULAG camp-prison system in 1956. The liquidation of Soviet human geneticists during the pre-war period explains why medical and particularly human genetics lagged behind in the rapid development of genetics after 1965.

The problems of human genetics were discussed only very briefly. In the first Soviet textbook on general genetics, written by Professor M. E. Lobashov and published in 1967, attention focused on the pattern of human chromosomes and immunogenetics. In only one chapter did Lobashov really repeat the old 'clichés' on the bourgeosie's misuse of human genetics. Through human genetics, he claimed, this had tried to "justify the exploitation of one class by another, antagonism between different nations and creation of the racialist theories". Lobashov also made some very positive statements about eugenics and considered that previous errors in this branch of human genetics did not necessarily indicate that it could not be developed into a useful field of research. Lobashov's view was that individuals in a human population, although equal in the social sense, have different genotypes and far from identical intellectual possibilities. These inherited differences had to be taken into consideration by educational training programmes so that the individual's potential could be released and developed.

At the same time, I. T. Frolov, philosopher and senior official of the Department of Science of the Central Committee of the Communist Party of the USSR, published a small booklet entitled *Methodological Problems of Genetics*. His main points were designed to prove that the collapse of Lysenko's pseudoscience would not in any way diminish the importance and validity of Marxist dialectic-materialist philosophy for biology and natural sciences in general. Lysenko simply did not use this philosophy correctly; he falsified and distorted both biology and dialectical materialism.

With his philosophical criticism of Lysenko's ideas, Frolov attempted to present the Marxist background to modern classical genetics, biochemical genetics and evolution. Human genetics were not mentioned in his essay at all, as if they were not part of the problem; the rehabilitation of genetics stopped short, neither ideologists nor scientists being able to analyse human genetics. Nobody wanted just to acquire knowledge from abroad, but all the roots of Soviet human genetics had been completely destroyed, and as yet nothing could appear from the rubble.

Development inevitable

The logic of science and the practical needs of medicine, however, made the development of human and medical genetics in the USSR inevitable. The Academy of Medical Sciences started to consider establishing a research institute in the field, and the leading figures of genetics and biology, none of whom had real practical knowledge of human or medical genetics, started discussing their possible task.

Initially, the discussions were very peaceful and pragmatic with nobody, quite understandably, wanting to take the lead. Among those taking part, Academician Nikolai Dubinin, Director of the newly-established Institute of General Genetics, was considered to be the main authority. His expertise lay in the field of *Drosophila* genetics. The next most prominent but most popular of the geneticists was Academician Boris Astraurov. He was elected the first President of the Society of Geneticists of the Soviet Union, which was established in 1966, and an expert on silk worm genetics. He established the Institute of Developmental Biology which excluded research on human genetics.

Of the others actively involved in the discussions, Professor Dmitry Beliaev, Director of Novosibirsk Institute of Cytology and Genetics, had experimented in the genetics of rabbits and some rodents. The man most excited about human genetics, Dr Vladimir Effroimson, was a silk worm geneticist whose very wide knowledge of medical genetics (he published the first Soviet book on medical genetics in 1963) was purely theoretical. The other members of the discussions came from similar backgrounds: Professor S. J. Alikhanian was Director of the Institute of Bacterial Genetics and Selection; A. A. Prokofieva-Belgovskaja and N. P. Bochkov were cytologists; N. P. Timofeev-Resovsky and R. L. Berg, drosophilists.

Although the project to establish the research institute on human genetics was generally supported, its organisation and priorities were not established in the meetings and discussions sponsored either by the Medical Academy or by the newly-created All-Union Society of Geneticists. The peaceful but not yet very productive discussions, however, were suddenly sharpened by Dubinin, who, like Frolov one year earlier, published a small popular book under the almost identical title, *Some Methodological Problems of Genetics*. The essay strongly politicised the discussion and opened up a new division among geneticists who only recently had represented a strong and friendly joint front against Lysenko.

Dubinin, introduced by the publisher as "a famous geneticist who had great influence on the development of Soviet and world genetics", started his essay in the usual demagogic style of his former Lysenkoist opponents. The methodological principles of the modern natural sciences, he claimed, had been founded by Lenin in his work *Materialism and Empirio-criticism* (1909). This had eternal importance for the further development of philosophy and all natural sciences, genetics in particular. After a short description of the history of modern genetics, with more than generous attention to Dubinin's own contributions and frequent quotations from Marx, Engels and Lenin, Dubinin unexpectedly made a statement on human genetics, differing little from Lysenko's approach to the field.

> "In research on human genetics the main task is to realise that the human race during its development excluded itself from the evolution of other animals . . . Now social factors only—class struggle, industrial, cultural and scientific progress—determine human evolution. Anthropogenesis is completed and ended by the appearance of consciousness. Now social factors are primary and biological factors secondary". (p. 60).

At first this statement did not look

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out of place. Nobody considered Dubinin an expert on human genetics and his colleagues and friends tried to explain to him that genetic laws and principles apply equally to all animals and that man is no exception. Very quickly, however, it became clear that the statement quoted above was not accidental; it was part of a developed system of views which recognised the human race as a qualitatively different, new and final stage of animal evolution.

Two years later, in 1970, Dubinin published another book for the general reader called *Genetics and Human Future*. By this time his ideas about the qualitative biological gap between man and other animals had developed. He tried to show that genetic make-up in humans bears no relation to the formation of personality. According to Dubinin's theory, human development from birth is determined by "social inheritance", everybody having potentially equal intellectual capabilities. Social inheritance is determined by the character of society itself and so far only the Communist societies can create the favourable conditions for the full development of human potential.

This book provoked more open discussion. Both sides tried to keep it outside serious academic journals, mostly because the discussion was based on opinions, not on facts or research. But both Astaurov and Effroimson wrote critical essays which were published in the popular literary magazine *Novy Mir* in 1971. Frolov, then Editor-in-Chief of the journal *Voprosy Filosofii* (Problems of Philosophy), organised a round-table discussion on human genetics, also sponsored by the Advisory Research Council, on the Problems of Philosophy of Nature at the Presidium of the Academy of Sciences of the USSR. It was clear from the discussion which was published in *Voprosy Filosofii* in 1972, that Dubinin's view received very little support.

Narrow objectives

The new Institute of Medical Genetics, which was finally established in 1971 within the Academy of Medical Sciences of the USSR, did not have the organisation to make the study of all aspects of human genetics possible. It was given narrow objectives and a comparatively young director, Nikolai Bochkov, a medical cytologist. Bochkov's appointment was supported by Dubinin who expected him to be an ally in the personal confrontations which now made up the discussion.

In 1973 Dubinin published *Vechnoe Dyizhenie* (Perpetual Motion), a rather self-promoting autobiography. Reviewed very widely and favourably in the official press, it distorted the factual history of Soviet genetics and gave a very mild version of the Lysenko controversy. In it, though, Dubinin tried to reply to his critics and to formulate the main principles of his vision of human genetics.

> ". . . The attempts to whitewash eugenics and to use for this purpose new genetic discoveries had been made by B. L. Astaurov, A. A. Neifakh and M. D. Golubovsky. V. P. Effroimson developed wrong views about genetic determination of intellectual and personal characteristics of man. This trend started to be an ideological danger. Again alien ideology tried to poison the clear sources of our science. I tried to make an uncompromised denunciation of this ideologically harmful attitude. Alas, I met a small, but well united group of opponents; B. L. Astaurov, S. M. Gershenson, S. I. Alikanian, D. K. Beliaev who defended their wrong position. This worried me and proved that some old geneticists do not understand Marxist ideas about human race and principles of society which is the only factor determining the intellectual specificity of man . . ." (p. 434).

‘According to Dubinin, human development is determined by "social inheritance", which is determined by the character of society itself. Only the Communist societies can create the favourable conditions for the full development of human potential’

Dubinin also tried to develop the distinction between the genetic control of physiological and psychological characteristics of human individuals. Genes, according to his view, determine the programme of development, lifespan, some pathologies, expectancy of heart troubles, cancer and resistance to infections. The shape of nose and mouth, or height, are also inherited traits. However, personality and intellect, he claimed, depend not on genes but only on social environment and self-training; their possibilities are unlimited. As soon as man is able to think consciously, he isolates himself from the laws of biology. All "normal" persons are psychologically and mentally equal at birth and so there is no reason for taking the racialist approach when talking about the quality of human genotypes with regard to intellect.

> ". . . Let us remember the story of emigration from Russia of many representatives of bourgeois art, scientific and thechnological elite after the October Revolution. Some eugenicists considered this exodus as an irretrievable loss of valuable genes. But this was nonsense. New talents from grass roots through Revolution found their way to lead the country, the Party, to create new science, new art, to create the great Soviet Union! . . . This fact illustrates that the creation of personality does not depend on genes, but is induced by the information of social environment." (p. 426).

In 1974 Academician Boris Astaurov died from a heart attack—a great loss for Soviet biology. His death was probably hastened by strong political pressures induced by the defection of one of the senior research scientists from his institute. As director, Astaurov had recommended that this good research worker present a paper at an international conference in Italy. The man asked for political asylum and did not return to the USSR. In such cases, the official bureaucracy makes directors personally responsible. Special Academy commissions started to 'investigate' (with active participation by Dubinin) all aspects of the ideological and moral atmosphere of Astaurov's institute. Some senior members of his staff were demoted or dismissed, and Astaurov's health suffered.

Not long before his death, Astaurov, as a member of the Editorial Board of a popular scientific journal *Priroda* (Nature), recommended that it publish a translation of the article "Man as a Biological Species" by Professor Ernest Mayr, a prominent American zoologist and population geneticist. The article did not ignore the social aspects of formation of human personality, but it considered all characteristics and qualities of individuals to be a result of the interaction of genotype and environmental and social influences.

Mayr's article provoked interesting discussion in *Priroda;* several anthropologists and philosophers supported his views. Dubinin did not join in the *Priroda* discussion, but instead attacked Mayr's article in a paper published in a philosophy journal where he insisted that all the spiritual aspects of human life are outside biology or genetics. He labelled Mayr's synthetic, social and biological approach as "reactionary" and "racialist".

Reply published

The average publication time in Soviet journals, including popular scientific ones, is very long. Consequently a reply to Dubinin's article, written by D. K. Beliaev, was not published in *Priroda* until a year later. Beliaev, who in 1976 had already been appointed President of the 1978 International Genetic Congress in Moscow, was reluctant to acknowledge the seriousness of the controversy among the Soviet geneticists. He clearly explained, however, that he disagreed with Dubinin's simplistic explanations. Behaviour, he wrote, as well as many of man's intellectual abilities, certainly depend on social factors, teaching and training, but these factors act on a highly differentiated population and the main organ of mental activity—

the human brain—is not excluded from genetic laws and genetic diversity.

Dubinin published a new detailed article on the subject which appeared earlier this year in *Voprosy Filosofii*. It mostly repeated his earlier arguments, though it was more politicised. The ideas about the intellectual or spiritual aspects of human life became rather mystical, in spite of regular quotations from Marx, Engels and Lenin.

Through a complex of pseudo-scientific argumentation, Dubinin tried to state that the intellectual and ethical progress of *Homo sapiens humanis* is properly directed in one social system only. In any other social system there is no progressive evolution. Social systems create the real man after birth; the priority of genes before birth is not questioned. Good systems create good persons, bad create bad. From Dubinin's autobiography it is clear that he considers himself a good man whose psychology and intellect has been developed under the influence of the best social systems. "I am thankful for my fate . . . I do not want to live another life . . . " He is happy. During his 70 years of life, he never discovered that 30 years of Stalinist terror were not good for the creation of *Homo sapiens humanis.*

There is, however, at least one important factor for securing the happiness of *Homo sapiens logic*—freedom to do research, whether genetic, social or political. Using his position as the director of the main Institute of General Genetics, his influence as Consultant on Biology for the Central Party Committee and his membership of different commissions and groups, Dubinin is working hard to suppress by all possible means the development of genuine research in the field of human genetics in the USSR. Even now the Soviet Union is not involved in many important fields of research in human genetics. As part of medical science, human genetics now has modest conditions for research into several specially selected problems, mainly associated with health programmes. But as a part of general biology, evolution research or population genetics, human genetics does not really exist in the USSR.

In a great country with more than a hundred national minorities, several different races and many different cultural roots, human genetics is probably too hot a science to handle. ☐

ILL at ease

Judy Redfearn finds out about neutron research at the Institut Laue-Langevin and talks to its director

NEUTRONS have long been recognised as valuable tools for research. Their importance is still growing as new techniques are enabling them to find new applications in different branches of science. One of the world centres for neutron research is the Institut Max von Laue-Paul Langevin (ILL) in Grenoble, France. It is jointly run by Britain, France and Germany, and this year, when it celebrates its tenth anniversary, has seen the appointment of a British scientist as its director.

He is Dr John White, a physical chemist from St John's College, Oxford, who took over from Professor R. Mössbauer, a German, last March. He faced a pressing problem after he took up his new post: what to do with spent fuel rods from the high flux reactor, the cornerstone of ILL's neutron facility. ILL has been assured of a fuel supply since President Carter lifted his ban on the export of enriched uranium, but the freeze on reprocessing caused some worries. Just last week, however, it was granted a licence to import 12 fuel elements into the USA for reprocessing at ERDA's Savannah River plant in Georgia.

But President Carter's policy is not the only factor which may influence ILL's future. The lifetime of the high flux reactor and the value of all ILL's facilities to its member states are now being assessed to determine the institute's long term prospects after 1982, the earliest date at which the three member countries could, under the original agreement, revoke their commitment. An extensive study of the reactor has shown that it may yet have 20–25 years more life in it—"a tribute", says Dr White, "to the French and German engineers who built it". If further studies support this, a report on the institute's future, due in the autumn, should give a firm assurance that the reactor will continue operating into the 1980s.

How it will continue, however, is not yet certain. The report is expected to recommend whether or not it should go into a 'deuxième souffle'—a second stage involving the member states in another major investment to extend the facility's scope. Dr White believes that ILL will only survive into the 1980s if instruments and techniques are constantly up-dated. "At the moment we have got world leadership in this area —and we have got to keep it up."

A matter of satisfaction

ILL's future success will depend, as it always has, on how well it satisfies its visiting researchers. Unlike many other laboratories and research organisations, it is essentially a users' laboratory. Some 70% of the neutron-beam time is available for outside experiments, mainly from universities and institutes in the three member countries but also from many other parts of the world. Only 400 staff, of which 70 are PhD scientists, are employed under five-year contracts to keep instruments running. ILL's Scientific Council considers approximately 700 experimental proposals each year; at its last meeting in March, it reported requests for twice as much experimental time as is available.

Experiments originating in the member countries are funded by the organisations, or Associates, which run the institute: the Science Research Council in Britain, the Gesellschaft für Kernforschung in West Germany, and the Commissariat à l'Energie Atomique and the Centre National de la Recherche Scientifique in France. France and Germany signed the first intergovernmental treaty giving the go-ahead for the building of ILL in 1967. It was not until 1973 that Britain, having abandoned an idea to build its own intense neutron source, joined in, paying a share of the original capital investment. Routine operation of the high flux reactor, which cost FF240 million of the FF335 million spent on building the whole institute, began in 1972. It has almost completed five years of successful operation and most of the instruments designed around it

ILL

ILL's director, John White

have now been working for 2½ years.

The high flux reactor is possibly the most intense continuous neutron source in the world at the moment. It operates at 57 MW and contains a single fuel element of 9 g of 90% enriched uranium. Because such highly enriched uranium is contained within a small reactor core, the fission process is concentrated into a small volume and the resulting neutron radiation is very intense.

"If the first unique feature of this reactor is its high flux density", says Dr White, "the second is the interesting and varied sources of neutrons which surround the core". Neutrons straight from the reactor are all slowed down to thermal velocities as they pass through a vessel of deuterium at 300 K. Those emerging from a particular part of the reactor are then speeded up by a piece of hot graphite to form a hot source. Other neutrons pass through liquid deuterium at 25 K and are slowed down even further to form a cold source. But perhaps the most important feature of ILL's reactor is the great increase in experimental area created by conducting some of the thermal and cold neutrons through special conducting pipes away from the space surrounding the reactor, which is crowded with instruments.

It is the ways in which they interact with matter that have made neutrons valuable research tools. Four properties make slow neutrons particularly useful in the study of liquids and solids: their electrical neutrality, short wavelength, low energy and magnetic moment. Because they have no electric charge they can penetrate the atom's electron shell and interact with the nucleus. Their short wavelength—of the same order as the atomic diameter—means that they are scattered by a regular array of atoms in a crystal to form a neutron diffraction pattern. Their low energy—of the same order as the quanta of vibrational energy of the scattering sample—means the dynamics of solids and liquids can be studied by measuring speed changes in the scattered neutrons. So called magnetic scattering occurs when the magnetic moment of the neutron interacts with an atom's magnetic moment; it facilitates the study of the magnetic properties of solids and liquids.

ILL's high flux reactor: view from the top

ILL

Diversification

Physicists were aware of the value of the neutron's properties 20 years ago when they started using them for studies in solid state physics. But it is only over the past ten years that they have been used in chemistry; and in the past three to four years they have found applications in biology as well—last year saw the first international conference on neutrons in biology. Dr White says that ILL's curious instrumentation has helped it to make a unique contribution in some of the new areas of science, particularly in polymer chemistry, biology and nuclear physics. The structure of biological materials, for example, can often be revealed in the diffraction patterns of neutrons scattered from them.

The phenomenon which makes neutrons so potentially valuable in chemistry and biology is called 'contrast'. It can best be explained by analogy with light. A glass rod immersed in a liquid will be clearly visible provided that the refractive index of the glass differs from that of the liquid. If the liquid's refractive index is changed to be the same as that of the glass, the rod will seem to disappear. Similarly, a biological material can be immersed in a mixture of heavy and light water whose refractive index with respect to neutrons is the same as parts of the material. Those parts of the material will then make no contribution to the neutron diffraction pattern and will have been 'contrasted' out. This means, for example, that a neutron diffraction pattern could be formed from, say, the RNA molecules inside a virus, no contribution to the pattern being made by any other part of the virus.

Dr White believes that the recent branching out into broader areas of science has come from a happy interaction between the interest of the users of ILL and the instrumentation that was already there. Britain added to the creative atmosphere by bringing many problems to be solved; the French and German approach had always been to design instruments to find new ways of working with neutrons. The two approaches combined well to bring forward an expansion in the use of neutrons for research. As Dr White puts it, "The French are a bit Cartesian, they tend to think mathematically; the Germans are Leibnitzian; and the British tend to be empirical". But he does feel that ILL gains rather than suffers from the fusion of these three different scientific temperaments.

The institute is assured of a supply of fresh ideas not only because so many different nationalities work together, but also because there is a regular turnover of permanent staff. Although the average age of the staff, 34, means that ILL is a place for young people with good ideas, it does have one disadvantage. Each year the 2½–3% net growth in ILL's budget allowed for by the Associates is gobbled up as the predominantely young staff moves up the salary scale, leaving very little for building new instruments.

"At the moment we are only building one or so instruments a year, which is hopeless because the normal amortisation rate for such heavily used instruments as those at ILL is 10 years at the most. At the present rate of building, instruments will be 15 years old or more before they can be replaced". Somehow, ILL is hoping to find a way of subsidising its budget so that more instruments can be built.

Move under way

A move in this direction is already under way. Since March, Dr White's main scientific concern has been in establishing a source of ultra-cold neutrons which are just energetic enough to reach a height of 15 feet in the earth's gravitational field. They are totally internally reflected from quite common materials and so can easily be trapped for experimentation.

Perhaps the most exciting application they could have is in the field of neutron optics. Existing techniques for focusing neutrons, for example, are as crude as the techniques used by Newton 300 years ago to focus light. As yet there is no neutron lens but the ultra cold neutrons travel so slowly that they can be focused with a magnetic field and could be used to develop neutron-focusing techniques. Already a highly coherent neutron beam has been produced at ILL. It is by no means a neutron laser, but it does have some of a laser's properties which could be useful in neutron optic studies.

"The people who come to ILL have got particular problems that can be solved no other way than by neutrons", says Dr White. "Even five years ago, we wouldn't have believed that neutrons would have the sensitivity to detect the things they now do; but with the high flux and the specialised instruments at Grenoble, we have seen hitherto unobservable things". When the spallation neutron source—a pulsed source of very hot neutrons just approved in Britain—is built at the UK's Rutherford Laboratory, he claims that Europe will house the world's most sophisticated neutron facilities. □

Assessing the 'greenhouse effect'

A report on the climatic effects of energy consumption was made public last week. Colin Norman reports from Washington

If the world's burgeoning demand for energy is met by burning increasing amounts of fossil fuels, there could be significant changes in the Earth's climate, lasting at least a millennium, a committee of the National Academy of Sciences warned in a report made public last week. Though the report is hedged with uncertainties and qualifications, the committee reckons that if the use of fossil fuels continues to increase at present rates, average global temperature could rise by about 6°C over the next 200 years, with potentially dire consequences for agriculture and fisheries.

The report, prepared by a committee headed by Roger Revelle, a noted ecologist who holds joint appointments at Harvard and the University of California, constitutes the first major attempt to assess the validity and potential consequences of the so-called "greenhouse effect", which may result from loading the atmosphere with carbon dioxide by burning coal, oil and gas. The theory is that carbon dioxide in the atmosphere will absorb heat radiated from the Earth's surface, causing an increase in global temperature, which in turn could trigger a variety of climatic changes.

The greenhouse effect was first postulated several years ago, but the theory has been controversial and the magnitude of its potential impact has been the subject of considerable debate. The Academy committee acknowledges that the data supporting the theory are incomplete, but the evidence is nevertheless strong and "the implications warrant prompt attention". Though the committee looked into other potential climatic impacts of energy use, such as the direct addition of heat to the environment and increasing the amount of particulates spewed into the atmosphere, it found that the carbon dioxide effect is likely to be far the most significant and it "may be the primary limiting factor on energy production from fossil fuels over the next few centuries".

On the basis of a number of climatic models and several years of direct measurements, the committee calculates that the concentration of carbon dioxide in the atmosphere has increased by between 11% and 13% from the beginning of the industrial revolution to the present. It may increase by 25% by the turn of the century, double by the year 2050 and reach between four and eight times its pre-industrial level in 200 years' time, if use of fossil fuels continues to grow.

The effect of such a rise in carbon dioxide concentration would be a 6 °C increase in average global temperature over the next 200 years and temperatures at the poles would be expected to increase even more, the committee states. In addition, precipitation is likely to increase and there could be severe regional changes in weather patterns.

It is important, however, to note the basis for those predictions first, the projected increase in carbon dioxide concentration is based on the assumption that use of fossil fuels will continue to grow as the industrialised countries switch from oil and gas to coal to meet their energy needs. Another key assumption is that per capita energy consumption will increase and that population will rise to a level of 10 billion toward the end of the twenty-first century. Those projections could be greatly affected by policies to curb the environmental impact of strip mining, by deliberate attempts to convert to other energy sources, by efforts to curb energy demand, and by policies to restrict population growth. The report's conclusions are therefore essentially based on an extension of present trends.

The projections are, however, founded on some key measurements of carbon dioxide concentrations at sites in Hawaii and the South Pole over an 18-year period during the 1960s and early 1970s. The measurements, made by Charles D. Keeling of the University of California at San Diego, are the first positive confirmation that carbon dioxide levels are increasing. They indicate that carbon dioxide concentration increased by about 5% over the period, and they suggest that about 40% of the carbon dioxide released into the atmosphere had remained there. The committee concludes that about 40% of the carbon dioxide released during that period was taken up by the terrestrial biosphere and the remaining 20% was taken up by the oceans. The projections of a doubling of carbon dioxide concentration over the next century and a four-to-eight fold increase by the year 2050 are essentially an extension of those calculations and measurements.

The link between increasing carbon dioxide concentrations and increasing global temperature is based on a computer model of the Earth's climate which the committee acknowledges is "imperfect in a number of significant ways" but is "the most complete yet devised". The model predicts a 3 °C increase in the temperature of the lower atmosphere for each doubling in carbon dioxide concentration, and a

TVA appointment

President Carter last week gave a clear signal of his intention to set a new course for the giant Tennessee Valley Authority (TVA) by nominating S. David Freeman, an advocate of energy conservation and solar power, to fill a long-standing vacancy on TVA's board of directors. Freeman, who first came to prominence when he directed the Ford Foundation's multi-million dollar Energy Policy Project, is also expected to become chairman of the three-member board when the present incumbent, Aubrey Wagner, retires next year.

Established in the Great Depression as part of Franklin Roosevelt's New Deal, TVA has developed into the largest electric utility in the United States, serving an area the size of Great Britain. It was created primarily to raise the economic level of one of the poorest regions in the United States, but energy supplies dominated operations. It is the largest single purchaser of strip-mined coal, and has had a bitter battle with the Environmental Protection Agency over its policy of dispersing pollutants from its power plants through tall smokestacks. TVA has also embarked on a massive nuclear programme, with plans to have as many as 17 nuclear reactors in operation by the mid-1980s.

Critics have contended that TVA's poor environmental record, and its relative neglect of alternative sources of energy, are a far cry from its original goal. Carter has echoed such complaints. Now he has indicated that he would like the authority to recapture some of the innovative spirit which predominated during its early years. Freeman is at present an assistant to James Schlesinger, Carter's chief energy adviser.

Colin Norman

7% rise in average precipitation.

The effects of such an increase in temperature could be serious. The committee notes that there would be a poleward shift in the world's agricultural zones, marked changes in the location and extent of arid and semiarid zones, and potentially serious changes in regions of precipitation. For the north American breadbasket, for example, the effect could be to push the corn belt northwards off the productive soils of the midwest and on to poorer Canadian soils. The impact on the oceans could be even more severe. A warming of the surface layers could be expected, which would reduce vertical circulation, thereby reducing the biological productivity of the oceans because it would depress the circulation of nutrients. There would also be a significant shift in the location of the major fisheries.

A five-degree warming of the upper 1,000 m of ocean water would also raise the ocean level by simple expansion by about 1 m. Melting of sea ice would augment the rise in ocean level. As for the potential impact on the polar ice caps, the committee states that "in the present state of understanding, it is impossible to forecast what might happen to the Greenland and Antarctic ice caps". Since temperatures over the ice caps would be expected to remain below the freezing point, however, "melting at or near the surface of the ice probably would not occur". But increased precipitation associated with increased levels of carbon dioxide could lead to substantial increase in ice thickness, which in turn could cause severe stresses at the base of the ice caps and surges and slides of ice into the sea. "If these surges resulted in destruction of the west Antarctic ice cap, there might be a corresponding rise in sea level of about 5 m within 300 years", the committee notes.

The report does not consider in detail the potential impact of global warming on regional weather patterns, climatic variability and shifts in ocean currents because of the difficulty predicting such effects.

The committee's chief recommendation is that a major research effort should be instituted, on a worldwide basis, to address some of the major unknowns in the climatic models and measurements on which the projections are based. Such an effort could cost between $20 and $100 million a year, Revelle suggested last week.

The report's chief impact, however, is likely to be on discussions of energy policy. The committee notes that if the potential for climatic change is substantiated, it may be necessary to reverse the trend of consumption of fossil fuels. The committee adds: "In the face of so much uncertainty regarding climatic change, it may be argued that the wisest attitude would be laissez faire. Unfortunately, it will take a millennium for the effects of a century of fossil fuels to dissipate. If the decision is postponed until the impact of man-made climate changes has been felt, then, for all practical purposes, the die will already have been cast". ☐

Jupiter success

THANKS to an intense lobbying effort by space scientists, university administrators and Governor Jerry Brown of California, the Jupiter Orbiter Probe, a key space science project, has survived a strong challenge in Congress. The House of Representatives last week rejected a recommendation from its powerful appropriations committee that the project should be killed, and voted funds to begin building the spacecraft next year. Since the Senate had already approved the project, the House vote constitutes the final go-ahead.

The Congressional debate over the Jupiter project provides an object lesson in the politics of big science. It also demonstrates the power that the scientific community can muster when it unites behind a major project.

Long considered a top priority space science mission, the Jupiter Orbiter Probe will involve the launch in January 1982 of twin spacecraft to orbit Jupiter and send scientific instruments into the planet's atmosphere. The total cost is expected to be close to $300 million. Though the project has been on the drawing board for several years, this is the first year that the National Aeronautics and Space Administration (NASA) has asked Congress for funds to begin building hardware.

NASA officials were not anticipating much opposition to the project from Congress, and they consequently received a shock earlier this year when a House appropriations subcommittee, chaired by Edward Boland of Massachusetts, recommended that all funds for the mission be deleted from NASA's budget. The subcommittee's action, which was later upheld by the full appropriations committee and the House itself, was based on the fact that NASA was also requesting funds to begin building the Large Space Telescope. The subcommittee said that it would be willing to sanction only one major space science project, and it opted for the telescope.

Once the subcommittee's vote against the project was made known, the scientific community mobilised to reverse the decision. A flood of letters and telephone calls descended on members of the Senate appropriations committee in an attempt to persuade the Senate to keep funds for the Jupiter project in its version of the NASA budget bill. The lobbying was successful, and it was then widely expected that when differences between the House and Senate versions of the budget bill were ironed out by a House-Senate conference committee, the Jupiter project would emerge with sufficient money to meet the launch schedule. But further problems arose.

House members of the conference committee refused to compromise on the project, insisting that it must be dropped in order to provide more money for higher priority efforts. Senate conferees, similarly, insisted that the project be allowed to proceed. To break the deadlock, the matter was referred back to the House for a separate vote last week. The lobbying was at its most intense shortly before the House vote.

The Jupiter Orbiter Probe is the only planetary project up for approval, and NASA officials thus argued that if it were killed there would be a major hiatus in planetary research. Abandoning the project would result in breaking up teams of scientists and engineers who have been working on planetary programmes, and continuity from one project to another would be lost, NASA officials argued. Moreover, since there would not be another suitable launch window until 1987, denial of funds for the project this year would effectively put Jupiter out of reach for another five years.

The chief political asset for the project, however, was the fact that it will be based at the Jet Propulsion Laboratory in California, a state with a large Congressional delegation and considerable political muscle. Alarmed at the prospect of losing a $300 million federal project, Governor Brown telephoned a number of Congressmen before last week's vote and urged them to support the project. As a result, the California delegation united with other supporters of the project, and the opposition of the appropriations subcommittee was easily overcome. The vote in the end was 131 to 280.

Colin Norman

BRITAIN

● Concorde supporters can take heart at the news that British Airways is to use the plane to fly twelve batches of generators of a newly-developed radiopharmaceutical across the Atlantic to waiting patients with lung complaints at Johns Hopkins Medical Unit, Baltimore, and the Washington Hospital Center. Doctors at Johns Hopkins took delivery of the first batch last week, and are reportedly delighted with it.

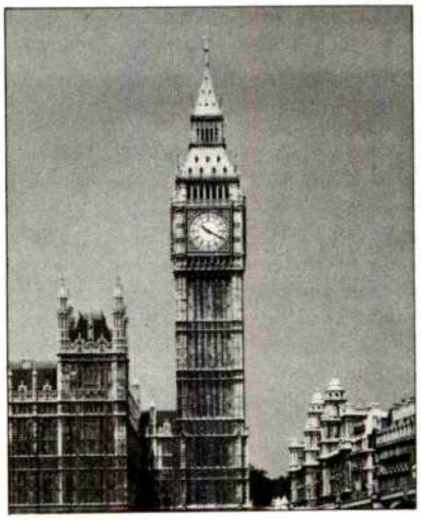
The new radiopharmaceutical, krypton-81m, helps doctors diagnose lung diseases. It was developed about two years ago by researchers at the Medical Research Council Cyclotron Unit at Hammersmith Hospital in London. It cannot be flown across the Atlantic subsonically because rubidium-81, the generator from which the krypton-81m is obtained, decays so quickly; it would be too weak to be used by the time it arrived. But Concorde cuts the transatlantic crossing time from 8 to about 3½ hours, and if twice the normal requirement of rubidium-81 is loaded onto the plane, sufficient radioactive strength remains after the journey for up to ten hours of clinical use.

It is krypton-81m's short half-life which makes it so useful. At 13 seconds, it is the shortest-lived radioisotope in medical use. The patient is positioned in front of a gamma camera and breathes in air containing a trace of the radioactive krypton which decays rapidly inside the lungs. The gamma camera records the regional arrival of air, enabling doctors to see immediately which parts of the lung are receiving no air. Previously only total lung volume could be measured, using long-lived isotopes.

At Hammersmith, krypton-81m has been used to monitor progress and response to different treatments. By itself it is not a good diagnostic technique, but in conjunction with standard perfusion techniques, which record blood supply to the lungs, it provides unique information for the diagnosis of such lung diseases as pulmonary embolism, asthma and chronic bronchitis.

● The good thing about the UK National Coal Board's (NCB) activities last year was the £27 million profit it made, said its chairman, Sir Derek Ezra, when the 1976–77 annual report was published last week. What wasn't so good, he said, was the fall in production and productivity.

This apparent paradox has arisen three years after the introduction of Plan for Coal, a scheme set up in 1974 which would allow expanded exploration and research. Sir Derek puts 1976–77's profit down to achievements under the Plan as well as increased sales, a 10% increase in open-cast mining output and economies of operation.



The most notable short-term research success was the development of the in-seam miner, which won the Queen's Award for Technological Achievement. It has mechanised some manual tasks and has improved the working of face ends and the opening of new faces. Short-to-medium term mining research programmes aim at further automation. Fluidised bed combustors to improve coal utilisation are being developed for commercial use. They are already available on a small scale, but have yet to be developed for use in power stations.

The long term hopes for achievement in coal research, however, go hand in hand with expectations for coal in meeting future energy demands. If coal is to become a more important energy source as oil supplies dry up, the NCB must increase production, find new coalfields and develop ways to convert coal to chemical feedstocks, gas and liquid fuels. Under the long term plan, Plan 2000, research aims at completely automated mining, especially of deep or remote seams inaccessible to miners. One method enjoying moderate success is the removal of coal which has first been liquidised underground, and a multidisciplinary group has explored the long term possibilities of such methods with research establishments in the UK, USA and West Germany.

All in all the NCB spent £20.8 million on research in 1976–77, about 0.7% of gross turnover. Of the different research categories—mining, utilisation, medical and others—mining received the lion's share. The trend is expected to continue this year.

● Professor Thomas Symington, director of the Institute of Cancer Research (ICR), has announced his retirement two years before it was expected. The decision was made mainly on personal grounds and its suddenness will leave the Institute temporarily without a director. Professor Leonard Lamerton of the Institute's Biophysics Department will be director for the time being until a substantive director is appointed.

The Institute of Cancer Research, consisting of the Chester Beatty Research Institute in London, the Pollards Wood Research Station at Chalfont St. Giles in Buckinghamshire and several Departments on the Sutton, Surrey, site of the Royal Marsden Hospital, has an annual budget of about £3 million: half of this comes from the Medical Research Council (MRC), £650,000 from the Cancer Research Campaign and the residue from legacies' interest and grants from overseas. The ICR has had to face a fall in the MRC's block grant and the removal of a £200,000 allocation to the animal facilities. Symington told *Nature* that this was a temporary problem and had no influence on his decision to retire.

When Symington became director seven years ago, the Institute needed reorganisation to rid itself of its less effective research staff. The MRC insisted on converting much of its funding from a block grant into support of specific programmes and projects to be accounted for on a regular basis. By most accounts the Institute under Symington came to withstand that kind of scrutiny. He leaves wishing only that he could have unified the various parts of the Institute on the campus of a medical school.

● Pro-nuclear Labour Party activists could face a problem when the party gathers in Brighton for its annual conference in the first week of October. Of nine resolutions on energy, not one has a good word for nuclear power—and not one fails to mention nuclear power. Three resolutions appear on nuclear weapons, and just one on defence generally.

BRITAIN

Awkward decision

In an embarrassing and controversial decision, the UK Medical Research Council (MRC) last week agreed to "run down" its Demyelinating Diseases Unit in Newcastle. Chris Sherwell reports

ON THE face of it, the MRC's decision is in line with its overall policy regarding research units, namely that they last as long as the directors to which they are attached. A glance at the two most recent MRC annual reports, for example, shows a sizeable turnover, with two or three units a year appearing and more disbanding. The director of the Demyelinating Diseases Unit left over a year ago. So without a new director a run down and redeployment of the unit's 33 staff, if not closure altogether, was always on the cards.

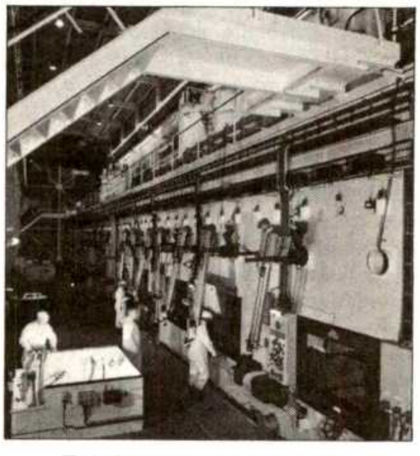
The demonstration outside MRC headquarters on the day the decision was taken, however, suggested that this particular case was different. And so it is. Partly this is because a well organised and highly unionised staff at the unit was resisting the MRC, an example of a phenomenon that the Council is only just beginning to accommodate. The more important reason is that the MRC has set such store by its multiple sclerosis (MS) research effort, of which the unit is a part. Not to be able to find a suitable director to take over the unit, which is the problem the MRC says it has faced, has made for many red faces. Some concerned lobbying from MS-related organisations has added to the controversy.

So too has the unit's stormy history, which finds its best expression in the circumstances surrounding the departure of E. L. Field as director in 1973. His controversial work is still debated, some say partly because the failed efforts to repeat his work have never been published. Henry Wisniewski became director in October 1974 but left in June last year for what were described as personal reasons. Work inspired by his animal model continues; this involves the development of a chronic relapsing form of experimental allergic encephalomyelitis which in important ways mimics MS.

Whether this would have remained a promising course of research, particularly without Wisniewski, is now a matter for speculation. But his departure brought more administrative problems, for he had been difficult to find. In an effort to keep the unit going the MRC cast round again for another director. One man who was invited declined, in spite of what the MRC calls "considerable material inducements". Several other candidates applied, including at least one from the unit itself. None fitted the bill. Advertisements which broadened the scope of the job to include dementias had not helped.

In May the MRC took a decision in principle to run the unit down. The Council met last week to consider an appeal from the staff, but the outcome was almost a foregone conclusion. The statement confirming that the decision would stand expressed great regret. A formal statement giving detailed reasons is expected this week. Whether this will try to explain how and why no director could be found to take the job is unclear. But in the meantime the MRC will probably be hoping that the unit it is now running down might one day start again. □

Material aid



Decanning Magnox fuel (BNFL)

BACKGROUNDER

"LOST!", the *Sun's* front page headline boldly proclaimed. "Enough plutonium to make fourteen A-bombs. Finder please return to Tony Benn". It was one way—a popular daily's way—of reporting what was perhaps last week's non-story in Britain. Most other papers recognised it for what it was and gave it less prominence. But they still faced the problem of explaining that there was no problem.

The occasion was the publication by the UK Atomic Energy Authority (UKAEA) and British Nuclear Fuels Ltd (BNFL) of data on nuclear materials accounting in Britain. The figures seemed to show that, since 1970, 96.8 kg of plutonium had gone "missing" from Windscale and 97.6 kg of highly enriched uranium from Dounreay. These are the sums of annual inventory differences—of arithmetical differences between the physical inventory and the book inventory of nuclear materials coming into, moving through and leaving any particular site or part of a site.

The annual inventory difference is known as MUF—material unaccounted for. And it is never zero. In these nuclear-fuel fearing days, the idea that plutonium or enriched uranium cannot be accounted for is, well, explosive. After all, if material always *seems* to be missing, how is anyone to know when it really is missing? But it does not always seem to be missing; sometimes it seems to be growing. And for the same reasons that the UKAEA and BNFL are sure that no one has given them material illicitly, they are sure no one has taken it either. Those reasons are to be found in the security measures they adopt, of which MUF is simply not an integral part.

So what is MUF? Essentially it is a recognition of the need to account for valuable materials—a need admittedly highlighted by the first reports three months ago that a shipload of uranium disappeared in 1968 between Antwerp and Genoa and by demands from such groups as Friends of the Earth for details of nuclear materials accounting in Britain and elsewhere. But accounting has always been there; and the techniques are apparently improving. What is new are the figures themselves.

In Britain the accounting balances are struck on a quarterly or monthly basis. Their minimal value as an aid against diversion might improve if, as is said to happen at some US plants, a balance is struck once a shift. But the MUF concept was not designed for this purpose.

The figures will move closer to zero only as built-in sources of error are reduced. These include inaccuracies in, for example, estimating the amounts of plutonium in irradiated fuel elements. Estimates of plutonium at other stages are also fraught with difficulty. No one is expecting the figures to reach zero.

MUF figures justify few fears or hopes. But if the accounting wasn't there, it would have to be invented; and most people would rather have the figures calculated.

IN BRIEF

R & D statistics

Total expenditure on R & D at colleges and universities in the United States increased by 9% last year, about 2% above the rate of inflation, according to a series of figures released last week by the National Science Foundation. Within the total, however, spending on basic research rose by only 5%, less than the rate of inflation, while support for applied research rose faster than inflation.

The overall proportion of R&D funds in universities and colleges devoted to basic research has declined from 77% in 1970, to about 68% this year, the figures suggest. Earlier this year, a study of the health of academic science indicated that declining support for basic research has seriously eroded the quality of research in some institutions.

MAFF's chief scientist

After five years as Chief Scientist at the UK Ministry of Agriculture, Fisheries and Food, Sir Charles Pereira will be retiring at the end of November. Professor B. G. F. Weitz, Director of the National Institute for Research in Dairying at the University of Reading, will succeed him. Professor Weitz will inherit responsibility for identifying R & D needs and for the department as a 'customer' under the customer/contractor arrangements for applied research. He will also have £42 million at his disposal for R&D in 1977–78.

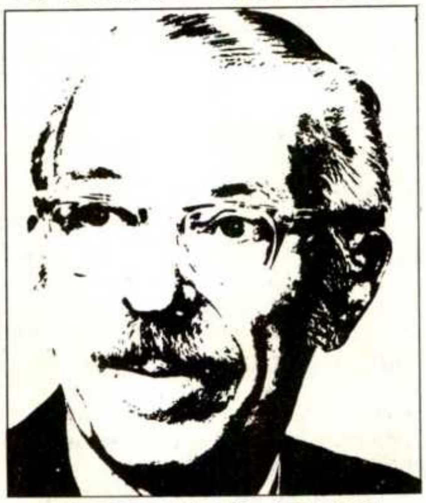
Professor Weitz's research interests have included the development of Brucella vaccines, the action of tetanus and diptheria antitoxins and the immunological aspects of tropical diseases transmitted by insect vectors.

CERN's saving

Figures which emerged at the June session of the CERN Council in Geneva confirmed a SF50.1 million saving (at 1970 prices) on the super proton synchrotron (SPS) programme that will end in 1979. The SPS programme is now estimated at SF1,099.9 million. The Council also agreed to prolong until August 1980 the agreement CERN has with the European Southern Observatory; the 1978 budget for this is estimated at SF618.7 million.

From the beginning of next year the new leader of CERN's experimental physics division will be Dr Erwin Gabathuler (43), from Britain. He has been project leader, European muon collaboration, since 1974. Before that he was responsible for the Daresbury-based programme at CERN.

COSPAR in Israel



THOMAS H. JUKES

ACRONYMS are a modern nuisance, but all of us make use of them to some extent. COSPAR means the Committee on Space Research of the International Council of Scientific Unions, and this mouthful refers to an international scientific organisation concerned with fundamental research carried out with the use of rocket-propelled vehicles. COSPAR functions as a sort of scientific mini-UN, and holds annual meetings in various member countries. This year, after considerable behind-the-scenes give-and-take with the Soviets, the invitation of Israel as host had finally been accepted. There were symposia on instruments for space astronomy, on travelling interplanetary phenomena, on Viking experiments and on global food information systems. There were also, as usual, meetings of various 'working groups', all of which are recorded in the Book of Chronicles of the Goings-on in Israel (also known as the abstracts and proceedings of the twentieth COSPAR meeting).

I have been regaled many times with accounts of visits to the Holy Land, starting with Mark Twain's *Innocents Abroad*, but I found that to see Israel for the first time is a deeply cultural and emotional experience. The land stands once more at the pinnacle of a climactic episode of her history. Who can deny that the rest of the world owes something to the Jews, the Israelis, in the light of what has happened in the past 50 years? But how can I phrase these ideas adequately? I am subdued before the oratory of the great, spanning the millennia, from Elijah to Abba Eban, and by the words of the Irishman who reminded us that the tables of the Law were graven in the language of the outlaw.

I must urge you to visit the museum of Yad Vashem in Jerusalem. We approached it through a small avenue of trees, each of which is dedicated to a Gentile who helped the Jews during the era of the Nazi holocaust. After the fire comes a still, small voice. Replicas of the Yad Vashem museum should be built throughout the world, especially in the Soviet Union, so that all may see its contents, which vividly depict what happened in Germany and in the countries occupied by Hitler, 1939–1945.

The Dead Sea is a grand object-lesson in comparative toxicity; useful in discussions of the subject with consumers who are nervous about poisons in their food. Drinking moderate quantities of Dead Sea water is fatal, because of its high content of magnesium (14%), sodium (7%), and potassium (4%) chlorides. A drop splashed in the eye causes intense pain. Yet these salts, in lower concentrations, are essential to life. The most crucial activity of all cells, protein synthesis, depends on ribosomes, and these need magnesium ions to maintain their integrity and function. Neither can we exist without sodium, postassium and chloride ions.

The contrasts in Israel are startling. We drove, on a modern highway, out of a brand-new town with sprinklers watering the flowering oleander bushes. Soon we were in barren, rolling country where Bedouin Arabs herded goats beside tents whose design had not changed for centuries, camels who sniffed the evening, and donkeys who serenaded it under high-tension power lines. Back to the coast we rolled through the ancient city of Jaffa, and to the new University of Tel Aviv for an outdoor evening reception. Here a professor introduced the dean, in a short speech that included a gentle reminder of budgetary needs for his department. The dean, after the manner of his kind, welcomed us warmly, but made no promises about the budget. We drank the wine, labelled 'Product of Israel, Cabernet Sauvignon'. It was just like California, where most red wines seem to be labelled 'Cabernet Sauvignon'. Vive la France, vive le COSPAR!

correspondence

Torture

SIR,—In June, Amnesty International published a monograph containing the results of three research studies conducted by its Danish Medical Group[1]. The studies concerned modern methods of torture. This group, which came into being in October 1974, is the first of its kind, and its pioneer work will serve as a pattern for similar groups in other countries.

One of the reasons that this group was formed was the disclosure that in certain countries doctors have cooperated in inventing new, sophisticated ways of torture that can be used without leaving any visible evidence. To be able to prove that such methods have been used, medical experts are needed. Ingenuity in this field seems to be unlimited, but with every new revelation of torture methods, one can only hope that public opinion will have a preventative or at least restraining effect on those using them.

Thanks to the initiative of the Danish doctors, Amnesty International will form a 'Medical Advisory Board' which will encourage the forming of research and action groups in other countries and coordinate their work. Such groups have come into existence in the USA, Sweden, Holland and within the near future there may be a similar group in Britain.

Forming research and action groups is one way of helping to implement the 1975 declaration against torture that was accepted in Tokyo by the WMA General Assembly. In this declaration doctors are admonished to abstain from participating in any physical or psychological torture of prisoners. The 'Declaration of Tokyo' is an important document in a time when torture unfortunately is a matter of common occurrence in a number of countries in different parts of the world.

A doctor must refuse to participate in torturing a prisoner, but should he not also actively oppose torture? The Amnesty groups of doctors are of that opinion. As an example of what official medical organisations can do, the Swedish Medical Association has faced the question of acting on behalf of individuals or minorities that have been subjected to political persecution, torture and other inhuman treatment. As a rule, Amnesty International has initiated these actions, but the association has also been approached independently by its members.

Naturally, the possibilities for a medical association to have any real influence are very limited. In cases where the documentation is good, however, it has been our experience that protests to respective heads of state or other appropriate authorities can benefit prisoners and give positive results.

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[1] *Evidence of Torture: Studies by the Amnesty International Danish Medical Group.* Pp. 40. (Amnesty International Publications: (London). Originally published in Danish in *Ugeskrift for Laeger*, 2 May 1977.

A combination derivation of the proton–electron mass ratio

SIR,—Atkin[1], Bastin[2] and Kilmister[3] have recently shown that a discrete algebraic theory readily generates the structures and cardinalities of the kind contemplated by Eddington[4]. In particular, Kilmister has pointed out that algebraic structures having 10 and 136 components are inevitably perpetuated by such a combinatorial approach.

It is well known that Eddington attempted to use these two numbers to account for the proton–electron mass ratio. He proposed that this ratio be identified with the ratio of the roots of the quadratic equation.

$$10m^2-136m+1 = 0 \qquad (1)$$

This quadratic root ratio is 1847.6 and was an adequate fit to the known experimental data for determining the proton–electron mass ratio in 1933, when this proposal was first made. When it became clear, however, that the experimental ratio was close to 1836, Eddington, with rather intricate physical justification, added factors to his original equation to approximate the new result. This procedure has rightly been criticised. As Kilmister puts it: "This is almost certainly the wrong way to go about things. We ought to realise that if we have any tenable theory at all on this point, we have one that agrees approximately with the results. In order to obtain better agreement, we need to recast the whole theory, not to patch it up by multiplications by constants whose values differ very little from unity".

Actually, Eddington could have recast his theory by dispensing with his notorious quadratic altogether. He had to do something physically plausible with the key cardinal numbers 10 and 136 to derive 1836. From a combinatorial point of view (with one eye open to the physics) the most straightforward procedure is the following:

if we have 136 things to choose from and need to choose 2 at a time (one for the proton, one for the electron presumably) we will have (136)(135) = 18360 ways of choosing. If we then have to distribute these ways of choosing into 10 parts (for the 10 gravitational potentials perhaps) we end up with the desired number

$$P_2^{136}/10 = 1836. \qquad (2)$$

This is close enough to the observed ratio of 1836.109 to suggest something stronger than mere coincidence.

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[1] Atkin R. H. & Bastin Ted *Int. J. theor. Phys.* 3, 449–466 (1970).

[2] Bastin E. W. (Ted) in *Quantum Theory and Beyond* (edit. by Bastin T.) 213–226 (Cambridge University Press London 1971).

[3] Kilmister C. W. *Sir Arthur Eddington* 269–271 (Pergamon Press Oxford 1966).

[4] Eddington Sir A.S. *Relativity Theory of Protons and Electrons* (Cambridge University Press London 1936).

Proposed terminology

SIR,—George S. Kell (23 June, page 665), is to be congratulated and applauded for inviting discussion of his proposed new terminology rather than incorporating it willy-nilly in a scientific paper. If other scientists had been as modest and careful then I doubt if the jargon would have been saddled with misleading terms like charm and colour. I, for one, am not charmed by the strangeness of the particle physicist's use of colour but I am sure that many viewers of a recent BBC science programme actually believe that quarks come in pretty shades of red and green. They have colour have they not? Physicists who indulge in purloining common adjectives to describe properties which bear no relation to the original meaning of the words should pause, reflect and be glad that at some time past they had not labelled some property 'gay', as in gay particle physicist.

George Kell has made a good try at avoiding such whimsy but should glance at a dictionary of geology where he will find 'psammitic' already in use, thereby pre-empting the root of his proposed terminology.

WILLIAM WELSH

University of Aberdeen, Scotland

news and views

The Twin Paradox revisited

from Tom Wilkie

THE muon may be a curious particle, but the study of its properties is a fruitful one (see *Nature* **267**, 582; 1977). The accurate determination of the muon lifetime, reported in this issue (page 301), has also provided a stringent check on Einstein's Theory of Special Relativity. As an added bonus, it sheds light on the 'Twin Paradox', gives an upper limit to the granularity of space-time, and tests CPT invariance of the weak interaction. The precision of the experiment is to within 0.1%, presently the most accurate test of Special Relativity using elementary particles. It was carried out using the CERN Muon Storage Ring which was built to measure the anomalous magnetic moment of the muon and so test Quantum Electrodynamics (QED). The present experiment is doubly welcome, in that it continues the desirable trend for elementary particle experiments of very high precision, and in that it makes good use of already existing apparatus. Too often in the past, particle physicists have been content only with gross trends in their data; too often also, there have been demands for very expensive equipment to perform a single experiment. In the autumn the Ring will be adapted to accelerator physics, and will start a new life investigating the exciting field of stochastic and electron 'cooling' of proton beams (*Particle Accelerators* 7(4); 1976).

As a species of massive electron, the muon is a charged particle with intrinsic spin $\frac{1}{2}\hbar$; it therefore has a magnetic moment when placed in an external magnetic field. It is unstable; in the laboratory it decays into an electron and two neutrinos, in approximately 2.2 μs. Recently, however, there has been intense theoretical speculation that, within the context of non-abelian gauge theories, the muon may decay directly into an electron and a photon (thus violating muon conservation number). These suggestions (*Phys. Lett.* **67B**, 303 & 309; 1977) have not yet been verified experimentally. The conventional muon decay pattern has a characteristic 'sickle' shape corresponding to

$$\begin{array}{l} \pi^- \rightarrow \mu^- + \bar{\nu}_\mu \\ \quad \hookrightarrow e^- + \nu_\mu + \bar{\nu}_e \end{array}$$

This distinctive pattern is seen when the pions are stopped in a bubble-chamber, and allowed to decay at rest. In the CERN experiments, however, the pions are injected into a 14-m diameter storage ring, and allowed to decay in flight. Because this decay violates parity, the produced muons are spin-polarised and their observed decay products, the electrons, are emitted along the direction of the muon spin. It is this ability to measure the muon spin direction which allows an accurate value of the anomalous magnetic moment to be found. The theory of QED, which essentially marries Quantum Mechanics and Special Relativity, predicts certain corrections to the naive, non-realistic value of the magnetic moment. The beautiful experiment done at CERN gave a triumphant measure of these corrections (Bailey *Phys. Lett.* **68B**, 191; 1977)

$$a_\mu\ (\text{expt.}) = (1{,}165{,}922 \pm 9)\times 10^{-9}$$

$$a_\mu\ (\text{theory}) = (1{,}165{,}921 \pm 10)\times 10^{-9}$$

Although this result may be considered a very complete confirmation of QED as the most accurate physical theory ever devised, it is at best an indirect test of Special Relativity. Because the muon is unstable it can be regarded as a subnuclear 'clock', and used to measure the time dilation effect predicted by Einstein. If two identical clocks are in uniform relative motion and an observer, considering his clock as stationary, compares the time measured by the two clocks, he will see the 'moving' clock losing time as it recedes from him. However, an observer with the 'moving' clock will consider himself to be stationary, and observe the first one to be going slow. This time dilation has aroused some passion (*Nature* **255**, 519; 1975). The so-called 'Twin' or 'Clock Paradox' arises when the 'moving' clock is later returned to the stationary one; it is indeed seen to have measured a smaller time-interval. But why could we not have regarded the 'moving' clock as stationary, and expected the first observer to have aged less? The resolution is that the 'moving' system has to change its velocity (that is, undergo acceleration) to return to the stationary one; the two are not equivalent, inertial frames in the sense of Special Relativity.

The existence of cosmic-ray muons at ground level supports the idea of time dilation; for, if the muons' lifetime is not lengthened during flight, they would all decay in the upper atmosphere. There have been more precise experiments using the line-of-flight decay of accelerator-produced pions and kaons (*Nucl. Phys.* **48B**, 343; 1972; *Phys. Rev. Lett.* **34**, 1244; 1975). The advent of high-precision caesium atomic clocks has made the macroscopic test of time dilation possible. Hafele and Keating (*Science* **177**, 166; 1972) simply loaded such a clock on to a commercial jet airliner on a round-the-world trip! Although reporting a positive effect, the experimental errors were substantial.

A different approach (*Phys. Rev. Lett.* **4**, 165; 1960) uses the Mössbauer effect to examine the 'Twin Paradox'. Photons from ^{57}Fe decay at the centre of a rotor enter a resonant absorber on the perimeter, and so are prevented from registering in an external, stationary counter. When the rotor is spun rapidly, the count rate goes up as the moving absorber is no longer in resonance with the stationary emitter—its time has been dilated.

Similarly, the muons circling in the CERN storage ring at 99.94% of the speed of light closely fulfil the role of moving clocks. To move in a circle they must undergo a centripetal acceleration, that is, they are in a non-inertial frame, and so they mimic the moving twin. We would expect the CERN muons to remain younger than their stay-at-home brethren. It is indeed observed that the moving muons live longer in agreement, to one part in 10^3, with the predictions of Special Relativity. The stationary twin's time-scale is given by observation of

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muonium decay (*Sov. Phys. JETP* **40**, 811; 1974).

One of our basic theoretical assumptions is that of CPT invariance: the physical properties of particles and anti-particles should be identical, apart from their charge. It is reassuring that the CERN group should have accurately verified this also.

But one of the most interesting consequences of this experiment straddles both Special and General Relativity. Any test of Special Relativity is, ultimately, a test of the homogeneity of space-time. Is it possible that space itself is quantised; that there is a fundamental distance below which we cannot probe? There has been some theoretical speculation that the existence of miniature black holes would entail such a fundamental distance. The CERN experiment puts an unambiguous upper limit ($\sim10^{-15}$cm) on the granularity of space-time. In so doing, it places a lower limit on the mass of any black hole. □

Atmospheric carbon dioxide and forest clearance

from Peter D. Moore

THE build up of carbon dioxide in the atmosphere is now firmly established, although its consequences are still a matter for debate. The frequently quoted figures from Mauna Loa in Hawaii demonstrate a rise in atmospheric CO_2 from levels of 313 p.p.m. in 1958 to 327 p.p.m. in 1975. It is generally accepted that the bulk of this rise is due to the injection of CO_2 into the atmosphere as a consequence of the burning of fossil fuels. It is also likely that the oxidation of carbon contained in living (mainly plant) biomass and in soil organic matter and litter has contributed to the rise. Farmer and Baxter (*Nature* **247,** 273; 1974) for example, have published data which implies a considerable increase in atmospheric CO_2, perhaps as much as 20 p.p.m., during the first two decades of this century, and they considered that this could be accounted for by the oxidation of non-fossil organic carbon.

On the other hand, one might expect the raised atmospheric levels of CO_2 to result in a higher gross production on the part of photosynthetic plants. CO_2 is the limiting factor for rates of photosynthesis and raised CO_2 levels are widely used to enhance the growth of greenhouse crops. It is not unreasonable to hope, therefore, that global carbon fixation is increasing as a function of CO_2 concentration. Such an hypothesis is difficult to test, however. Hall, Ekdahl and Wartenberg (*Nature* **255,** 136; 1975) attempted an analysis of the Mauna Loa data which was designed to isolate CO_2 fluctuations due to changes in the biospheric reservoir of carbon. They estimated the month-by-month variation in CO_2 output by industry in the Northern Hemisphere and used the curves produced to correct the ambient CO_2 record over the period 1958–1972. Making allowance for oceanic uptake (estimated at 42%) they could then obtain values for the overall photosynthetic and respiration of the biosphere by comparing the differences between the succeeding seasonal peaks and troughs in the atmospheric CO_2 concentration. Their conclusion was that there had been no basic change in the pattern of biosphere metabolism. An alternative way of interpreting their data would be to postulate that any increase in production has been matched by an increase in respiration, or its chemical equivalent, biomass oxidation by combustion.

Bolin (*Science* **196,** 613; 1977) has recently discussed the influence of changes in land biota on the carbon cycle and he also emphasises the influence of forest clearance and agricultural development on the atmospheric carbon load. According to his calculations the atmosphere contains about 690×10^9 tons of carbon (327 p.p.m.). Over the last decade the input from fossil fuel combustion has averaged 4.0×10^9 ton yr^{-1}, but at the same time there have been changes in the biosphere which will also have led to a net input of carbon to the atmosphere. Bolin estimates these rates at: deforestation and fuel wood production, 1.1×10^9 ton yr^{-1}; changes in organic matter in the soil, 0.3×10^9; less the drain on the atmospheric carbon level resulting from reforestation, estimated at 0.3×10^9. Thus the overall net injection of carbon into the atmosphere due to human activity averages about 5.1×10^9 ton yr^{-1}.

Observations on atmospheric carbon suggest that only about 40% of this remains airborne, the rest being absorbed by the oceans plus (in Bolin's opinion) a small loss of carbon from the atmosphere created by the regrowth of vegetation following clearance.

There are several points concerning the clearance of forests and their replacement by agricultural systems which must be considered carefully. First, there is the extent of forest cover on our planet; Bolin suggests that dense forest covers about 30% of the Earth's land surface and contains about 90% of the Earth's living biomass. Obviously, clearance of forest results in an initial movement of carbon from a living reservoir to the atmosphere, since most forest clearance is accompanied by the burning of waste timber, and most wood used for construction or pulp will be converted into CO_2 after a lag phase (which will be very short in the case of pulp).

Although the forests represent a major carbon reservoir, it would be wrong to consider them all as an efficient carbon sink. Most natural forest vegetation is at or near a climax or equilibrium state. Biomass is at or close to the maximum attainable within the existing constraints of climate, which means that the gross production of the system will be matched by the combined respiration of autotrophs and heterotrophs (herbivores, carnivores, detrivores and decomposers) within the system (see Odum *Science* **164,** 262; 1969). Thus the climax forest is in a no-growth situation and its carbon uptake will be equalled by its carbon loss. Even fairly mature forests which have not yet attained climax conditions may be past their peak in terms of net ecosystem production.

Thus, to act as an efficient sink for atmospheric carbon, an ecosystem should ideally have a high net ecosystem production rate. The net primary production rate is not necessarily a good index of net ecosystem production. According to Odum net ecosystem production (biomass growth rate) reaches a peak in mid stages of succession.

If one were to consider forest clearance purely from the point of view of global carbon balance (which is, of course, equivalent to wearing ecological blinkers), it represents the creation rather than the destruction of a carbon sink in that it revitalises successional processes. It raises atmospheric carbon, however, by converting biomass carbon to CO_2 and by accelerating the decomposition of soil organic matter and plant litter.

The question must be raised whether one could use a fast-growing terrestrial biomass to compensate for the industrial injection of fossil carbon into the atmosphere. Some of the possibilities in this sphere have recently been considered by Dyson (*Energy* **2,** 287; 1977). Dyson concentrates upon the use of fast growing tree species and also wetland plant species as possible implements for atmospheric CO_2 control. For

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example, the American sycamore (*Platanus occidentalis*) has a production capacity equivalent to 750 tons of carbon km^{-2} yr^{-1}. At this rate one would need 7×10^6 km^2 to be planted with the tree in order to compensate fully for rising global atmospheric CO_2. Dyson claims that the United States could provide 7×10^5 km^2 of suitable land, but he fails to take into account the loss in CO_2 consumption resulting from replacement of low grade forest currently occupying such areas. Even more important is what you do with the crop once it reaches maturity. The essence of atmospheric CO_2 control is to lock up the carbon in an alternative, permanent reservoir. Sadly, organic carbon is only too readily oxidised and returned to the atmosphere as CO_2. It could be retained as a forest biomass, but pressure on land is unlikely to permit this.

A natural ecosystem, with an additional built-in carbon reservoir is the peat-forming system. But the bulk of the world's peatlands occur in high latitudes, are generally low in nutrients and have low productivity. International Biological Program data collated in *Primary Production and Production Processes, Tundra Biome* (edited by Bliss & Wielgolaski, Dublin, 1973) show that most tundra mires have annual productivities of only about 40–100 g m^{-2}. The raised mires of Britain have average peat accumulation rates of only about 4–10 cm per century (Hibbert & Switsur *New Phytol.* **77,** 793; 1976). Thus these systems cannot be regarded as very efficient carbon sinks. The more productive wetlands, such as *Typha* and *Phragmites* swamps and ecosystems dominated by the tropical water hyacinth (*Eichhornia crassipes*), present problems of carbon storage and the inhibition of decomposition.

One of the most critical limitations on any such use of biomass growth as a carbon sink is the energy expenditure necessary for the establishment, management, fertilisation, harvest and storage of the crop. Where energy consumption is still closely linked with CO_2 production one wonders whether any attempts at biological control of CO_2 will prove self-defeating. □

New quarks . . . now the good news

from D. Morgan

A conference on Particle Physics was held at Budapest on July 4–9, 1977.

THIS year's high energy physics conference held at Budapest provided a rich harvest of new experimental results powerfully reinforcing present trends in the subject. Most challenging was the report by Leon Lederman (Columbia University) of a new vector meson or group of vector mesons at 9.5 GeV which could very well signify a new form of hadronic matter. Great interest also attached to the first results of neutrino experiments on the new 400 GeV European SPS accelerator at CERN, Geneva. Both these and many other of the results reported at the conference bear on an outstanding theme of contemporary particle research—the structure of matter as seen through the weak and electromagnetic currents.

Present thinking is here dominated by a fusion of two sets of ideas—the unified gauge theories of weak and electromagnetic (current) interactions, and the quark parton model which extends the description of current interactions to the world of strongly interacting particles. The outcome is a very close analogy between the non-strongly interacting 'leptons' (the electron, the muon and their associated neutrinos) and the quarks from which the strongly interacting 'hadrons' are built. Before 1974, three types of quark sufficed to build the known hadrons. One of the great successes of the unified model was to predict the existence of a fourth type of quark, a prediction which was triumphantly vindicated by the discovery of the J/ψ family of 'new' particles. An implication of this whole way of thinking is that the best way of studying new types of hadron is through their weak and electromagnetic interactions. Hence the tremendous contemporary emphasis on neutrino experiments, on the study of e^+e^- annihilation and on the observation in hadron collisions of systems decaying to leptons. Objectives are to discover what are the constituents of matter, both lepton and quark, and how they couple to the weak and electromagnetic interactions: which of the alternative gauge theories is that chosen by nature. In a world with just four sorts of quark and four sorts of lepton, a simple possibility is afforded by the $SU(2)\times U(1)$ model of Steven Weinberg and Abdus Salam which successfully predicted the existence of neutral weak currents and which still gives a good description of neutrino phenomena, according to new data presented to the conference. There are nonetheless good reasons for exploring wider possibilities. In the first place, there exists increasingly secure evidence for a new heavy lepton τ of mass 1.8 GeV, which presumably has a companion neutrino ν_τ. The unified models favour schemes with an equal number of quarks and leptons, suggesting the existence of two more new kinds of presumably rather heavy quarks, nicknamed t='top' and b='bottom', and associated effects in the hadron spectrum. The current couplings of the extra quarks and leptons could simply replicate those of their lighter brothers or could take new forms. The place to seek such effects should be in neutrino experiments and in experiments probing the coupling to lepton pairs. Great interest had therefore attached to reports from a counter experiment at Fermilab of an anomalously large production of high mass hadron systems in high energy antineutrino reactions (the so-called 'high-anomaly'). This suggested not only the coupling of a new kind of quark (the bottom quark has suitable properties) but a new kind of coupling involving the opposite (right-handed) helicity states from those implicated previously. It is against this background that the new experiments are to be judged.

Neutrino experiments

The outcome of the new high-statistics CERN experiment reported by Jack Steinberger is completely to demolish the high-y anomaly in anti-neutrino reactions. Both for neutrinos and antineutrinos the new inclusive cross-sections very much reinforce trends previously perceived in experiments at lower energies and in deep inelastic charged lepton scattering. For proponents of new quarks this is the bad news.

New vector meson

The discovery of the new 9.5 GeV vector meson very much mirrors that of the J/ψ (3.1). Like the 3.1, the new 9.5 GeV effect is seen in the $\mu^+\mu^-$ spectrum from very high energy proton–nucleus collisions. The new data comes from an experiment designed with extreme care to maximise the sensitivity to small signals. This results in an extremely clean unambiguous signal for one or more resonances spanning a range of 1.5 GeV centred at 9.5 GeV, the signal being seen against a background which falls smoothly from 4 to 14 GeV. The resolution is insufficient to reveal narrow

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structures so a full analogy with the J/ψ and its associated spectrum is not established. Nonetheless, it seems extremely likely that we are getting a first glimpse of a further 'new' spectroscopy involving one or more new quarks—an exciting prospect for the new e^+e^- storage rings at Cornell, Hamberg, Novosibirsk and Stanford which will be capable of studying the new effects with much better resolution.

Many other new results and problems were discussed at the conference. From Stanford came news of a new recruit to the J/ψ spectrum of mass 3.77 GeV which provides rather striking confirmation of the accepted picture of these states. (It is of course upon this picture that the optimistic hopes for a further new spectroscopy depend.) There was also progress in the field of 'old' mesons with indications that the long awaited A_1 axial vector meson may at last have been established; also reported signals for new vector mesons in the 1–2 GeV range. A further intriguing contribution concerned very narrow effects seen in $\bar{p}p$ annihilation. There continues to be much speculation as to the structure of the weak interactions and of the lepton spectrum. With couplings as in the Weinberg–Salam model, one predicts specific parity violating effects in atomic spectra, effects which have been sought and not found in recent experiments at Oxford and Seattle. The consequent urge to examine alternative coupling schemes was for a time reinforced by unconfirmed reports of an appreciable $\mu \rightarrow e\gamma$ decay rate. It is natural to associate such lepton number non-conserving processes with non-vanishing neutrino masses. The consequent possibility of neutrino mixing was discussed at the conference by Bruno Pontecorvo. A further unresolved question in the field of leptons is posed by the observation of trimuon events of which a small number, mostly involving very large muon momenta have been observed by the same Fermilab group which saw the former high-y anomaly. Despite the current preoccupation with leptonic probes, the quark structure of hadrons is also being explored in wide angle hadron–hadron collisions notably at the CERN ISR from which came further information on the formation of hadronic jets. The outstanding problem for theorists is to understand the detailed mechanism for confining quarks in hadrons. Several interesting lines of work were reviewed but we still await solutions. A high spot of the conference was an evening lecture by Paul Dirac describing his epoch making discoveries of 50 years ago—an object lesson in how to find solutions, and in humility. ☐

Physicochemical hydrodynamics

from Stanley Bruckenstein

> The Levich Birthday Conference was held in Oxford on 11–13 July, 1977.

THE Conference, organised to honour Benjamin G. Levich's 60th birthday, aimed at assessing the present state of development of physicochemical hydrodynamics and its future thrusts. Physicochemical hydrodynamics was the name coined by Levich in 1952 to describe problems related to the influence of fluid flow on chemical or physical transformations, as well as the influence of physicochemical factors on fluid flow. The ideas in Levich's 1952 book and the Soviet work underlying it, were for the most part, overlooked until 1962 when his monograph was translated from the Russian into English. Almost immediately, research in certain aspects of electrochemistry and fluid mechanics began to reflect the influence of the Soviet literature and the Oxford Conference is a measure of western progress and innovation made in the past 15 years in this interdisciplinary field. About 70 papers were presented in two parallel sessions, which were attended by more than 100 chemists, engineers and physicists. The conference concerned itself with six topics: physical transport, interface mechanics, interspersed phases, chemical hydrodynamics, electrochemistry and physics.

The use of controlled mass transport in experimental electrochemistry has made a tremendous impact. The principal convective–diffusion controlled electrode systems in use today are based on the rotating disk and rotating ring-disk geometries. Semitransparent disks are being used to detect electrode intermediates produced photochemically in the diffusion layer adjacent to the electrode surface (W. J. Albery, Oxford University). It has been demonstrated that the quantum efficiency for the photochemical generation process is readily determined from the angular velocity dependence of the current, and the technique is now being applied to investigate the mechanism and efficiency of photogalvanic cells based on dissolved, absorbing species.

Electrochemical photogalvanic cells represent a particularly attractive technology for solar power generation, since in principle they are free of some of the problems associated with solid state solar cells. There is no need to match the physical properties of the materials forming the junction in a solution cell and polycrystalline semiconductor electrodes have already yielded power efficiencies greater than 5%. However, liquid solar cells have their own unique problems, one of which is photocorrosion of the semiconductor. Rotating ring-disk and sinusoidally modulated rotating disk electrodes were used (B. Miller, Bell Laboratories, Murray Hill, New Jersey) to study polycrystalline cadmium sulphide semiconducting electrodes in $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ and S^{2-}/S_x^{2-} media. Details were given on the means for establishing the potential at which significant photocorrosion begins. The S^{-2}/S_x^{2-} system was found to be far less susceptible to photocorrosion.

The use of electrochemistry as a tool to study fluid dynamics is becoming more common. Non-newtonian fluid studies at rotating disk electrodes have led to the verification of an inversion of flow direction at a critical rotation speed. Also drag reduction in turbulent flow in the presence of poly(ethylene oxide) has proved especially amenable to study with the rotating disk and rotating ring electrodes (L. Viet, CNRS, Paris).

A rotating disk study of the chemical vaporisation of silicon (M. L. Hitchman, RCA, Zurich) from $SiCl_4$ and H_2 permitted the separation of transport and chemical processes, and the determination of the heat of activation for the silicon deposition process. This approach appears to hold considerable promise for the study of analogous gas–solid reactions.

G. K. Batchelor presented Levich's plenary paper. Levich, who requested permission to emigrate from the Soviet Union several years ago, was not allowed to attend the conference. This paper was a comprehensive review of the entire field and delineated problem areas. In the discussion of heterogeneous chemical kinetics, the necessity for uniform accessibility of a surface, or calculable mass transport rates, was stressed. The paradox of the existence of a rigorous statistical theory and several semi-empirical theories of

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turbulence was discussed. Levich remarked that, despite their obvious weaknesses, the semi-empirical approaches did permit successful calculations in very complex flow situations. There is need for a more detailed study of the structure of turbulence at rough walls. Levich believes that the phenomena of higher viscosity at the interface of two fluids is due to the presence of surface active agents, not a "surface viscosity". The mechanism of the breaking of bubbles in liquid media, ascribed to internal motions of the gas in the bubble is not yet confirmed by convincing experimental data. In his discussion of statistical systems (a stochastic and disorderly flow regime in space and time) a number of unsolved problems were discussed, including that of rising bubbles in a liquid, breaking of a gas stream through a liquid layer, and the process of heat and mass transfer in a system of bubbles. ☐

Isotherm limits ocean magnetism

from Peter J. Smith

THE linear marine magnetic anomalies which have provided such important evidence for seafloor spreading must result from magnetised material within the crust and, possibly, the uppermost mantle. To say that, however, is to state little more than the obvious, although to go beyond it is difficult. With insufficient direct information from, say, deep drilling into the ocean floor it is impossible to constrain the variables in theoretical models of the magnetised zone in such a way as to provide a unique solution, even when the model is made unrealistically simple. For example, the deceptive regularity of the anomalies themselves gave rise in the first instance to an overly simplified model comprising alternating blocks of normally and reversely magnetised rock; but the magnetisations and vertical thicknesses of the blocks could be varied independently within certain limits to permit a large number of viable block arrangements.

In early versions of the alternating block model, block thicknesses ranged up to at least 9 km. Later, however, Carmichael (*Can. J. Earth Sci.* **7**, 239; 1970) and others concluded that if the high magnetisations found in rock samples dredged from the ocean floor were typical of those throughout the blocks, the magnetic layer could be as thin as 0.4–1.0 km. Indeed, this view proved so popular that the most common block thickness used in anomaly modelling ever since has apparently been 0.5 km (with magnetisations in the range 8×10^{-3}–20×10^{-3} emu cm^{-3}). But as Kristjansson and Watkins (*Earth planet. Sci. Lett.* **34**, 365; 1977) now re-emphasise, no one should make the mistake of regarding this thickness as anything more than a "computational artifact". It serves a useful purpose in anomaly modelling but bears little relation to reality.

The reality is that the zone producing the observed anomalies is likely to be structurally complicated, chemically and mineralogically inhomogeneous, the product of emplacement within an active ridge zone of variable width, and affected by post-emplacement rotation. Such complexities are currently the subject of intense study, and models which attempt to accommodate them are already beginning to appear. Kristjansson and Watkins, on the other hand, have taken a more direct approach in seeking information about the actual thickness of the magnetic zone beneath the active central volcanic area of Iceland. This area is, of course, the landward continuation of the oceanic Reykjanes ridge; and although the magnetic anomalies are less sharp than those over oceanic ridges proper, they may still be used to determine a magnetic zone thickness. Thus if the zone has an average magnetisation comparable to that of the surface basalts (15×10^{-3}–20×10^{-3} emu cm^{-3}) it need only have a thickness of 0.5 km to permit modelling of the overlying anomalies. Alternatively, if the observed proportion of tuff and breccia at the surface is taken to obtain at depth, the net magnetisation is more likely to be 6×10^{-3}–8×10^{-3} emu cm^{-3}, leading to a magnetic zone thickness of about 2 km. Either way, the "computational" thickness is not very great.

But again the reality is different, at least if the magnetic properties of igneous samples from eight deep (up to 2 km) boreholes are anything to go by. The room temperature magnetic susceptibility, for example, is highly variable (depending to some extent on rock type) but shows no systematic change with depth up to the 2 km limit. And since the boreholes lie in regions of high thermal gradient, geothermal activity and recent volcanism, this is equivalent to saying that the susceptibility shows no systematic change within zones having present temperatures of up to about 300 °C. Outside the highly thermal areas, however, the 300 °C isotherm corresponds to a depth of about 4.5 km. The implication therefore is that beneath parts of the central volcanic area of Iceland the magnetic zone extends to depths of at least 4.5 km.

This is much greater than the greatest depth deduced from magnetic anomalies, confirming that the latter is indeed a fiction. It also suggests to Kristjansson and Watkins that to think in terms of a physically discrete magnetic layer of any thickness is fundamentally wrong—a point which needs to be made if only because some workers have already shown a tendency to translate a mathematical convenience into a sort of reality by thinking aloud in terms of a distinct magnetic layer with a base accessible in principle to sampling (for example, by the Deep Sea Drilling Project). The point that Kristjansson and Watkins wish to emphasise is that in their view there is absolutely no reason to suppose that the lower limit of magnetisation, which must exist, is governed by anything other than the Curie point isotherm. Or to put it another way: there is no reason to suppose that the material immediately below the magnetised zone is any different from that in the zone itself. The former is simply at a temperature high enough to preclude its being magnetised. ☐

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Magnesium and energy

from E. F. Emley

An international conference on Energy Conservation in Production and Utilisation of Magnesium was held at the Massachusetts Institute of Technology on 25–27 May, 1977. The conference was co-sponsored by the MIT Center for Materials Science and Engineering, the US Energy Research and Development Administration (ERDA) and the International Magnesium Association.

"IN the opinion of knowledgeable materials engineers, magnesium is a structural metal whose time has come". This opening sentence from the announcement set the tone of the conference, which set out under the guidance of M. C. Flemings to "create a meaningful dialogue among industrial, university and government personnel that will lead to energy con-

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servation in the production of magnesium and through its utilisation".

For many years the use of magnesium has been hindered by factors such as limited sources of supply of the metal and smallness of demand—about one fiftieth of that for aluminium—which in turn have prevented realisation in production of the benefits of scale. General structural and aerospace uses have tended to decline and the growth of demand has reflected increasing use for non-structural applications, such as alloying with aluminium and desulphurising steel. Realisation that magnesium is a non-strategic material (sources, including seawater, are inexhaustible and very widely distributed) coupled with the possibilities of energy saving in applications where weight or inertial forces are important, has brought nearer the time when new investment on a scale that could lead to cost reductions can be contemplated. Meanwhile, important improvements to both the electrolytic and the thermal reduction routes were announced at the conference, and these alone should lead to substantial reductions in energy consumption and production cost.

At the first session, J. A. Barclay (US Bureau of Mines) set the scene by calculating the relative total energy consumption for producing aluminium and magnesium, and suggesting directions in which improvements might be sought. Converted to kwh(t)/tonne, his figures were 71,500 for aluminium by the Hall-Héroult route and 105,000 for magnesium by the Dow seawater electrolytic process, with savings of 9% and 6% for the ferrosilicon (Pidgeon version) and carbothermic processes respectively. Comparative figures presented by P. Lugagne (Société Française d'Electrometallurgie) indicated a net energy consumption of 76,500 kwh (t)/tonne for the Magnetherm version of the silicothermic process, which includes allowances for the energy content of saleable by-products amounting to about 15% of the total. A similar energy credit would be available to the electrolytic process if starting from brine, by selling the by-product chlorine. Looking to the future he foresaw that in 1990 the energy requirements to produce 1 tonne of each metal would be virtually the same. In similar vein, projections by R. B. Stein (Bechtel Corporation) indicated that the price curves for virgin aluminium and magnesium could cross about 1985.

It was confirmed at the meeting that Norsk-Hydro will bring on stream next year new plant involving the dehydration of waste $MgCl_2$ brines from the German potash industry. This will give rise to 2.8 tonne chlorine per tonne of metal produced, and lead to a substantial net energy gain. Dow have an apparently similar development in hand which is expected to result in an energy saving of 30%.

A high spot at the meeting was the announcement that J. M. Avery, well known for his contributions to blast-furnace technology, had invented a continuous version of the Magnetherm ferrosilicon reduction process, operating at atmospheric pressure, and that agreement had been reached with the Arthur D. Little organisation to evaluate the process.

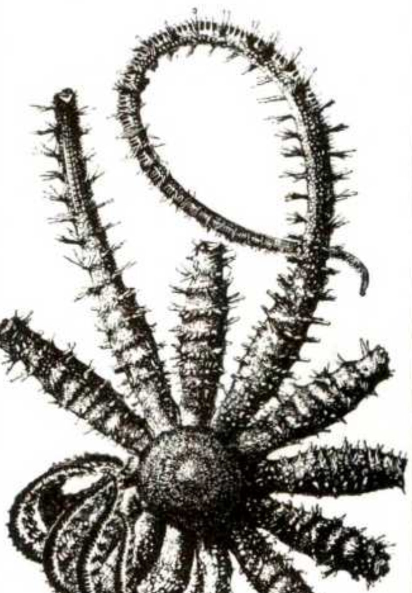
Following consideration of primary production, the conference turned to energy usage in fabrication, and the comprehensive data of S. L. Couling (Battelle), showed that the most favourable fabricating method, in terms of energy requirement, is hot-chamber die-casting. To deform equal volumes of aluminium or magnesium requires roughly the same energy, but less efficient furnaces and higher melt losses increase the energy requirements for wrought magnesium, as does the practice of pre-extruding stock for the extrusion process.

As regards growth of structural uses, M. S. Holland (Ford Motor Co.) saw the potential for magnesium in the automotive field as excellent, but not at the present price. With a primary Mg/Al price ratio of 2 : 1, as at present, the 1985 potential is seen as 5,000–10,000 t, whereas with a price ratio of 1.5 : 1 it could be as high as 100,000 t—more than the present day total US usage. Overall energy changes on replacing steel by aluminium or magnesium were further considered by J. P. Clarke and G. S. Kennedy (MIT) who found total energy requirement ratios close to 1.45 : 1 for each of the light metals compared with steel as unity when non-transport applications were considered. In automotive applications there can be an energy credit for the light metals owing to weight reduction and consequent fuel savings during the operational lifetime of the component, and this advantage is about 80% greater for magnesium than for aluminium.

The last day of the conference was devoted to R and D Workshops dealing with fabrication, properties, recycling, policy and, by popular request, primary extraction. Several workshops stressed the importance of price, and the need to get down to a price ratio of 1.5 compared with aluminium. Meanwhile the use of hot-chamber die-castings should be promoted, but there is need also for lower cost sheet and extrusions, for a metal-cleaning method without using flux, for a way of recovering metal from crucible residues, for ways of removing nickel, copper and tin from scrap, for reducing magnesium losses in the aluminium industry, for new approaches to sheet such as strip-casting and the pendulum mill, for avoiding the scalping and double extrusion of billet, for better or cheaper systems of protection against corrosion, for die-casting alloys with better elevated-temperature properties and reduced tendency to surface segregation, for stronger wrought alloys—the needs are many and the challenge to the technologist, whether in industrial, academic or government service, is great. □

NATURE

A hundred years ago



NEARLY a quarter of a century ago the celebrated poet and naturalist P. Chr. Asbjornsen, was dredging in the interior of the picturesque Hardanger-fjord, when, at a depth of about 200 fathoms, the dredge brought up a wonderful new star-like Echinoderm, quite unlike any form that had been up to that moment described.

In its unlikeness to most recent forms of star-fishes he saw its connection with certain fossil forms, and in its brilliant sun-like form he was reminded of the "Brising" which, according to ancient Norwegian tradition, was concealed by Loke in the abyss of the primeval ocean, but which had so long served as the ornament to cover the breast of the god Freya, and he gave the name of Brisinga to the new genus.

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articles

Measurements of relativistic time dilatation for positive and negative muons in a circular orbit

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The lifetimes of both positive and negative relativistic ($\gamma = 29.33$) muons have been measured in the CERN Muon Storage Ring with the results

$$\tau^+ = 64.419\ (58)\ \mu\text{s}, \quad \tau^- = 64.368\ (29)\ \mu\text{s}$$

The value for positive muons is in accordance with special relativity and the measured lifetime at rest: the Einstein time dilation factor agrees with experiment with a fractional error of 2×10^{-3} at 95% confidence. Assuming special relativity, the mean proper lifetime for μ^- is found to be

$$\tau_0^- = 2.1948\,(10)\ \mu\text{s}$$

the most accurate value reported to date. The agreement of this value with previously measured values of τ_0^+ confirms CPT invariance for the weak interaction in muon decay.

MEASUREMENT of the lifetime of a sample of radioactive material which is moving with a known velocity is a means of testing the so-called time dilation, or slowing down of moving clocks, predicted by the special theory of relativity. If, in addition the radioactive particles move in closed circular orbits then the conditions simulate those of the outward and return journey of the twin paradox in which, according to the theory, the journeying twin ages more slowly than the one who stays at home. In this situation it is also possible to search for any modification of the predictions of the theory due to acceleration.

A device which stores unstable elementary particles of high momentum is an excellent tool with which to carry out such measurements and here we report the results obtained with the Muon Storage Ring at CERN. Muons have a lifetime at rest of about 2.2 μs and decay into electrons neutrinos and antineutrinos

$$\mu^- \rightarrow e^- + \nu_\mu + \bar{\nu}_e$$

The decay electrons are readily detected. If the muon sample has a velocity v then the lifetime of the sample as measured in the laboratory is given by

$$\tau = \tau_0/[(1-(v/c)^2)^{\frac{1}{2}}] = \gamma\tau_0$$

where τ_0 is the lifetime for the particle at rest.

The previous Muon Storage Ring experiment at CERN reported[1] a value of the muon lifetime in flight for a relativistic factor $\gamma \simeq 12$, which agreed within 1% with the predicted value obtained by applying the above Einstein time dilation factor[2] to the measured lifetime at rest[3]. Here we report separate measurements for μ^+ and μ^-, with a γ factor of 29.33, which are an order of magnitude more precise and which show that the predictions of special relativity obtain even under accelerations as large as 10^{18} g and down to distances less than 10^{-15} cm.

In the latest Muon Storage Ring[4–9] muons of momentum 3.094 GeV/c ($\gamma \simeq 29.3$; $\beta = v/c = 0.9994$) circulate on orbits of 14 m diameter in a uniform magnetic field and a weak focusing electric quadrupole field[10]. The main purpose of the project was to measure the anomalous magnetic moment of the muon by observing the relative precession of its spin with respect to its momentum. The muons are born from pion decay

$$\pi^- \rightarrow \mu^- + \bar{\nu}_\mu$$

and by careful momentum selection in this process it is possible to obtain a circulating muon sample with high initial longitudinal polarisation. Decay electrons from the stored muons are detected in 20 shower counters inside the ring. By selecting only high energy electrons (forward going in the muon rest frame) it is possible to follow the muon spin precession since the decay electron distribution is assymetric with respect to the muon spin direction. The decay times are measured to high precision[11–13] so that the muon laboratory lifetime can be obtained by fitting the observed decay electron time spectrum which consists of an exponential decay modulated by the muon spin precession frequency (ω_a).

This spectrum is distorted by small systematic effects, however—notably the loss of muons from the trapping region before decay, the dependence of the background count rate, and the variation of the decay electron detection efficiency as a result of gain changes during the muon storage period. The resulting modification to the normal modulated exponential decay spectrum may be expressed as

$$N(t) = N_0[1+SG(t)+F(t)]\{\exp(-t/\tau) \times \times [1-A\cos(\omega_a t+\varphi)]+B(t)\} \quad (1)$$

The functions $SG(t)$ and $F(t)$ represent the variation of detection efficiency and muon losses, respectively, while $B(t)$ is the background. For μ^- data the background is independent of time and at the negligible level of $\sim 2\times 10^{-5}$, but becomes more important in the μ^+ lifetime data owing to stored proton contamination. The minimisation and measurement of these systematic effects are discussed in some detail below.

Muon losses

The muons are injected by the decay in flight of 3 GeV/*c* pions inflected into the ring[4-9]. They initially fill the whole available phase space; however, particle loss involves preferentially those muon orbits which approach closest to the material limits of the storage region.

To reduce these losses we manipulate the muon orbits in the first few microseconds after injection (the rotation period of the muons is 0.147 μs) so that the muons which pass within ~1 cm of the walls are removed. The remaining muons are left in the central region of the aperture and have a much smaller probability of being lost. This 'scraping' is achieved by applying asymmetric voltages to the electrostatic quadrupoles[10] used to focus the particles. A vertical electric field shifts the median plane downwards, and simultaneously a horizontal electric field (outwards on one side of the ring, inwards on the other) shifts the orbit sideways. The muons of large betatron amplitude then strike appropriately located aperture stops. The scraping voltages are turned off with a time constant of 10 μs, allowing the muon population to return adiabatically to the centre of the storage region.

When a muon hits an obstacle it loses energy and has a high probability of emerging on the inside of the ring. The muon losses are therefore monitored by placing a detector against the vacuum chamber on the inside of the ring, in a position similar to that occupied by the decay electron detectors. The muon detector consists of a series of four scintillation counters each preceded by two-radiation-length lead converters. Muons are discriminated from electrons on the basis of pulse height. A 'muon' is defined as a particle which, for all four counters, gives a pulse height equivalent to that of a minimum ionising particle, the acceptance ranging from 50% below to 50% above this central value. Measurements in an electron beam show that for the relevant range of decay electron energies, this condition rejects electrons by a factor of ~700. The muon detector is used to optimise the *n* value of the focusing field for minimum muon losses, to test the scraping system, and to measure the loss correction $F(t)$.

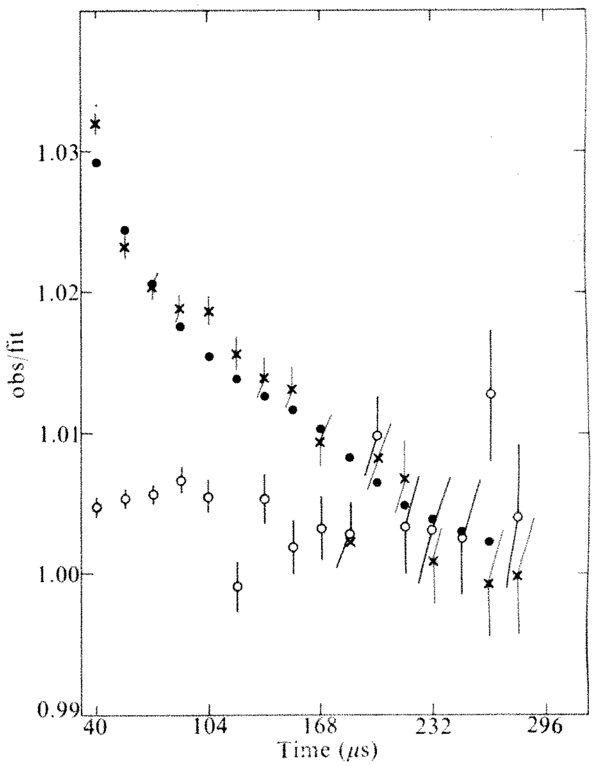

Fig. 1 Ratio of the decay electron time distribution (obs) to an exponential fit (fit) in the time range 300–650 μs. The *g*-2 modulation is included in the fit. The excess counts at early times over the extrapolated fit function show the effect of muon losses. ×, obs/fit no scraping. ●, Muon loss function as calculated from the muon detector time spectrum. ○, obs/fit with scraping.

In the optimum running conditions very few muons were lost after 100 μs. The above rejection ratio and the similarity in acceptance of the muon and electron detectors indicated that $\gtrsim 80\%$ of the 'muon' counts after this time were mis-identified electrons. This was confirmed with a small five-gap optical spark chamber placed behind the muon detector. Muons, identified by a straight track with four or five sparks, were found to form only 10% of the late time 'muon' counts.

The correction function $F(t)$ in equation (1) is given in terms of the lost muon time spectrum $M(t)$ (corrected for decay electron background) by the integral

$$F(t) = \frac{1}{\varepsilon\tau N_0}\int_t^{t_L} M(t)\exp(t/\tau)\,\mathrm{d}t \quad (2)$$

where t_L is the end of the storage period and where the factor ε is the ratio of the detection efficiencies of the muon and electron detectors; its value and hence the absolute calibration of the muon detector is obtained as follows. In data where the losses are relatively high (those taken without scraping in operation), $F(t)$ can be deduced directly from the decay electron time distribution. Comparison with the values obtained from equation (2) provides the calibration of the muon detector and checks that it samples the lost muons in an unbiased way at different times.

This procedure and the effectiveness of the electric scraping are illustrated in Fig. 1. The points (*a*) represent the fractional

Table 1 Results for the muon lifetime in flight (μs)

Run	A	B	C	D	E	F
Charge of μ	+	+	−	−	−	−
Starting time of fit	100	100	50	50	90	100
Uncorrected lifetime (statistical error)	64.406 (66)	64.295 (103)	64.444 (55)	64.336 (36)	64.366 (84)	64.278 (60)
μ-loss correction	0.008 (8)	0.008 (8)	0.013 (8)	0.012 (8)	0.009 (8)	0.008 (8)
p-background correction	0.006 (2)	0.044 (22)	—	—	—	—
Gain correction	0.022 (20)	0.016 (20)	−0.010 (20)	−0.002 (20)	0.033 (20)	0.020 (20)
Fully corrected lifetime (total error)	64.442 (69)	64.363 (108)	64.447 (59)	64.346 (42)	64.408 (87)	64.306 (64)

Table 2 Lifetime results (μs)

	μ^+	μ^-	Weighted average $\mu^+ + \mu^-$
Lifetime in flight (this experiment)	64.419 (58)	64.368 (29)	64.378 (26)
Lifetime at rest (this experiment)	2.1966 (20)	2.1948 (10)	2.1952 (9)
Lifetime at rest (previous best measurements, refs. 18, 19)	2.19711 (8)	2.198 (2)	—

excess decay electron counts at early time with respect to a fit to the unscraped data at late time ($t > 300$ μs). Taking 40 μs after injection as $t = 0$, gives $F(0) = 0.03$ for these data. The points (*b*) are those calculated from the lost muon data, and the time dependence of these two data sets are in excellent agreement. The points (*c*) are the early time excess counts for the scraped data obtained in just the same way as (*a*). The calibrated muon detector gave a value $F(0) = 0.001$ in this case, so the loss level at 40 μs after injection is improved by a factor of ~30.

For the late starting times chosen in the analysis, the muon losses contribute at most a shift of 0.02% in the lifetime, as can be seen from the entries in Table 1.

Proton background

For the μ^+ data there is another complication in that the time-varying electric field associated with scraping enables protons to be stored in the ring. (The incident π beam is unseparated.) These protons are lost with a characteristic time distribution and so give background in both the decay electron and muon detectors. By assuming that the muon losses are the same for $\mu^\pm$ with identical machine parameters, the time distribution of these lost protons can (by subtraction) be found from the counts recorded in the muon detector. The relative detection efficiency of the muon and electron detectors for these protons is established in a separate experiment where the electric field is switched off ~1 ms after injection. At this time (~15.5 τ) only protons are stored, and the ratio of counts seen in the muon and the electron detectors directly gives the efficiency ratio. It turns out that the background level in the decay electron counts due to these 'unstable' protons is low ($\sim 3 \times 10^{-3}$ at 32 μs after injection) so the resulting correction to the lifetime is small, as shown in Table 1. The contamination of the μ^- data by stored antiprotons was at a negligible level.

Gain effects

The largest systematic effect which must be considered is the change in the energy acceptance of the decay electron detection system as a function of muon storage time. This effect is due to gain changes following the injection into the ring of one or more ~10 ns wide bunches containing $\sim 10^7$ particles (for more details of the effect of high particle flux on the gain of photomultipliers, see ref. 14); it is minimised by blanking off the dynode chain of the photomultiplier during, and for a few microseconds after, injection

The system gain is measured as a function of time by means of light-emitting diodes (LEDs). The injected beam and all other conditions are the same as in normal data-taking. The signals from the LEDs are distinguished from decay electron counts by timing; a series of pulses are input to LEDs on the photomultipliers of the electron detectors at a number of fixed times throughout the muon storage interval. The output signal from each electron detector is split and input to two discriminators with a threshold separation of 3 dB. The LED pulse heights are adjusted so that all pulses fire the lower threshold discriminator and ~50% of the pulses fire the higher one, that is, the threshold of the latter is at the maximum of the LED pulse-height spectrum where the sensitivity to gain changes is greatest. A subsidiary calibration measurement of the pulse-height distribution for each LED-photomultiplier combination is made to establish the quantitative relation between the ratio of the counting rates output from the two discriminators and the gain change ΔG.

The gain curve is measured typically at 11 points between 7 and 580 μs for each counter, with a precision per point of ~0.1%. From the gain curves thus obtained, a counter selection is made to remove from the analysis counters with a steep gain variation. These counters are the ones which are struck preferentially by the injected beam. The mean gain curve of the remaining counters is typically flat to within ~0.1% over the whole muon storage interval. The mean gain curve $G(t)$ is multiplied by the factor $S = \Delta N/(N\Delta G)$, the fractional change of counting rate of the decay electrons per unit gain change, to give the correction function in equation (1). For the electron energy threshold chosen for the lifetime analysis, $S = 1.00 \pm 0.02$.

The error in the gain correction was estimated by repeated measurement under identical running conditions. From the consistency of the slopes of mean gain curves measured at intervals of both a few days and several hours apart, this error is conservatively estimated to be ~0.03% in τ.

Results

Values of the muon lifetime in flight τ were found by fitting the experimental decay electron time distribution to the six-parameter function of equation (1) using the maximum likelihood method and varying the six parameters N_0, τ, A, ω_a, φ, B_0, where B_0 is the time-independent part of $B(t)$.

The results obtained for the lifetime for the two μ^+ and four μ^- runs which were separately analysed are presented in Table 1. The uncorrected lifetime, and the corrections with estimated systematic errors due to muon loss and gain effects, are shown for each run. In addition, for the μ^+ data, the proton background correction and error are shown. The starting times quoted for the fits vary from run to run as, according to the beam intensity used, the muon detector took varying times to regain full efficiency after injection. In all cases the upper limit of the time range of the fit is 650 μs.

The weighted average values of the lifetimes in flight for $\mu^\pm$ together with the combined average are given in the first line of Table 2.

Fig. 2 Bunch structure in the Muon Storage Ring from 6–10 μs after injection. Each bunch corresponds to one turn in the ring. Such distributions are analysed to give the mean rotation frequency f_{rot} of the stored muons, and hence the mean γ-factor $\bar{\gamma}$.

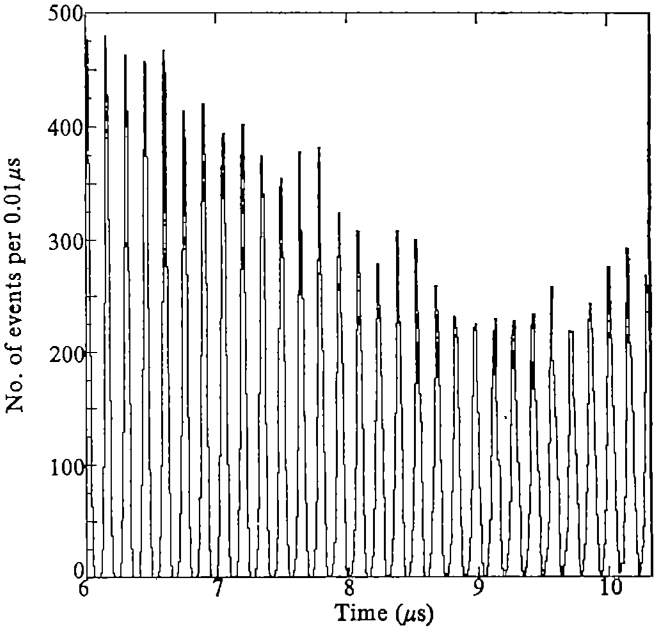

Table 3 Tests of relativistic time dilation from particle lifetime measurements. Values of $\tau_0 = \tau/\gamma$ are compared at different γ values

Particle	Refs	γ_1	γ_2	$[\tau_0(\gamma_1) - \tau_0(\gamma_2)]/\tau_0(\gamma_1)$ (%)	95% confidence limits (%)
$\pi\pm$	19–22	1.0	2.44	-2.5 ± 0.9	-4.3 to -0.7
$K\pm$	23, 24	1.0	3.38, 4.17	0.9 ± 0.3	0.3 to 1.5
K_S°	25, 27	1.63	15.2	0.5 ± 0.6	-0.7 to 1.7
K_S°	25, 26	1.63	20.2	0.2 ± 0.7	-1.2 to 1.6
μ^+	18 and this work	1.0	29.3	0.02 ± 0.09	-0.16 to 0.20

In the second line of Table 2 are given the corresponding lifetimes at rest τ_0 calculated using the Einstein[2] relation

$$\tau_0 = \tau/\gamma, \quad \gamma = [1-(v/c)^2]^{-\frac{1}{2}}$$

The average value of γ for the circulating muons is found by analysis[1] of the bunch structure of the stored muons (see Fig. 2) over a period of $\sim 40\ \mu s$ after injection. The mean rotation frequency $\bar{f}_{rot}$ is related to the average γ value $\bar{\gamma}$ by the expression

$$\bar{\gamma} = 2\lambda \bar{f}_p / g \bar{f}_{rot}$$

where $\bar{f}_p$ is the proton magnetic resonance frequency[15] (corrected to vacuum[16]) corresponding to the mean magnetic field, g is the g-factor of the muon, and $\lambda = \mu_\mu/\mu_p$ is the ratio of the muon and proton magnetic moments[17]. The result for $\bar{\gamma}$ is found to be

$$\bar{\gamma} = 29.327\,(4)$$

where the quoted error corresponds to an uncertainty of ± 1 mm in the mean radius of the distribution of circulating muons.

The last line in Table 2 gives the most accurate published values for the μ^+ (ref. 18) and μ^- (ref. 3) lifetimes measured at rest. Comparing the high precision value of the μ^+ lifetime at rest, $\tau_0{}^+$, with the value found in this experiment we obtain

$$(\tau_0{}^+ - \tau^+/\bar{\gamma})/\tau_0{}^+ = (2 \pm 9) \times 10^{-4}$$

At 95% confidence the fractional difference between $\tau_0{}^+$ and $\tau^+/\bar{\gamma}$ is in the range $(-1.6\text{–}2.0) \times 10^{-3}$. To date, this is the most accurate test of relativistic time dilation using elementary particles.

This result is compared in Table 3 with other limits on the validity of special relativity calculated from lifetime measurements given in the existing literature for $\pi^\pm$ (refs 19–22), $K^\pm$ (refs 23, 24) and K_S^0 (refs 25–27).

The entries in the table are the values of, and 95% confidence limits for $[\tau_0(\gamma_1) - \tau_0(\gamma_2)]/\tau_0(\gamma_1)$, where $\tau_0(\gamma_{1,2})$ are the lifetimes at rest as calculated via the Einstein relation using measurements on particles with mean γ values $\gamma_{1,2}$. The present experiment improves on the previously existing upper limits on violations of special relativity by about an order of magnitude.

The μ^+ measurement differs in one important respect from the other results quoted in Table 3. The $\pi^\pm$, $K^\pm$ and K_S^0 measurements in flight were performed in beams for which the decaying particle, regarded as a clock, was in an inertial (unaccelerated) frame. In the muon experiment, however, the particles are subjected to a constant transverse acceleration of 10^{21} cm s^{-2}. The muons perform a round trip and so when compared with a muon decaying at rest in the laboratory, simulate closely the so-called twin paradox which was already discussed in Einstein's first paper[2]. Other experiments[28,29] have confirmed the correctness of relativistic time dilation for clocks in circular motion to $\sim 10\%$ at low velocities (A similar conclusion follows from observations of the second-order (transverse) Doppler effect in the temperature dependence of the Mössbauer effect[23] and of the hydrogen maser frequency[24]). The present experiment is unique in its use of an ultra-relativistic clock ($\gamma \gg 1$) and its much greater precision $\sim 0.1\%$.

The possibility also exists that very large accelerations may modify in some way the internal enstitution of particles[32–35]. No such effects, in so far as they affect the particle lifetime, are seen in this experiment where the transverse acceleration is $\sim 10^{18}$ g.

Some authors[36,37] have suggested that just as special relativity breaks down at very large distances owing to gravitational effects, there may also be a breakdown below some fundamental distance α. Rédei[38] has calculated the effect of such a breakdown on the muon lifetime in flight, and finds a γ^2 correction to the Einstein formula

$$\tau = \gamma\tau_0\,(1 + 2.5 \times 10^{24}\,\gamma^2\alpha^2)$$

where α is in centimetres. From our value of τ^+ a limit can be set on α

$$\alpha \leqslant 9.6 \times 10^{-16}\ \text{cm} \quad (95\%\ \text{confidence level})$$

Assuming the correctness of special relativity, the μ^- lifetime at rest $\tau_0{}^-$ given in Table 2 is the most precise value so far reported. Comparing this value with the high-precision measurement of $\tau_0{}^+$ it is found that

$$(\tau_0{}^- - \tau_0{}^+)/\tau_0{}^+ = -0.00105(46)$$

Or, at 95% confidence level, the fractional difference of $\tau_0{}^-$ and $\tau_0{}^+$ is in the range -0.002 to -0.00013.

The limit set on this quantity, which should vanish[39] by CPT invariance of the weak interaction, is comparable with that set by the best direct measurement[3] of the ratio $\tau_0{}^-/\tau_0{}^+$.

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Rifting and subsidence on passive continental margins in the North East Atlantic

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Deep-sea drilling in the Bay of Biscay and the Rockall Plateau show that passive continental margins are characterised by the absence of seismicity and include continental shelf, continental slope and continental rise physiographic provinces, exemplified by the borders of the Atlantic Ocean.

HYPOTHESES postulated for the structural and stratigraphical evolution of passive margins are based on the available geological and geophysical data, and analogies drawn with present day margins thought to represent stages in margin evolution[1-5]. In the initial stages, rifting of the continent perhaps contemporaneous with regional uplift takes place[6]. When the continental crust is completely rifted apart, ocean crust begins to accrete at the axis of the diverging plates. Heat dissipation[7] through the cooling continental and oceanic lithosphere is thought to result in subsidence, permitting transgression. The subsequent development of the margin depends on the variable roles in both time and space of subsidence, sediment loading, climate and ocean circulation. For example, the lithology and volume of sediments may be influenced by global transgressions and regressions[8,9], although their effects, in turn, depend on the altitudes of the continents and their rate of subsidence.

The relationship between the subsidence history, the rifting environment and the unconformities observed beneath many passive margins is, in particular, poorly understood because of a paucity of relevant deep sea drilling data. We report here some preliminary relevant results from the holes drilled on the passive margins of the Bay of Biscay and the Rockall Plateau during Leg 48 of the International Phase of Ocean Drilling (IPOD) of the Deep Sea Drilling Project (Figs 1, 2, 3).

The Bay of Biscay and Rockall Plateau

The northern margin of the Bay of Biscay[10-14] is comprised of the wide Celtic shelf and a broad continental slope that is dissected by many canyons and broken by the Meriadzek Terrace. Detailed multi-channel seismic surveys made by the Institute Francais du Petrole, the Institute of Oceanographic Sciences and the Centre National d'Exploitation des Oceans, show thin Tertiary and Cretaceous sediments overlying tilted blocks and half grabens that trend sub-parallel to the margin and control the relief of the Meriadzek Terrace (Fig. 2). Previous geological and geophysical studies have suggested that the Bay of Biscay was formed by rifting in Late Jurassic–Early Cretaceous time and by spreading during Early to Upper Cretaceous time[10-18]. However, the relation between the spreading and the subsidence history of the margin is not known. The three sites drilled in Biscay (Fig. 2) form a transect from the upper slope (site 402A) to the lower slope (site 400A) within 40 km of the continent–ocean boundary at the Trevelyan escarpment[18].

In contrast to the Bay of Biscay, three phases of rifting and spreading isolated the Rockall Plateau microcontinent and formed its margins[19-23]. The first opened the Rockall Trough in Early–Late Cretaceous time, and the second, in Late Cretaceous time (76 Myr), opened the Labrador Sea, thus spreading the Greenland–Rockall plate away from North America. In the final phase, rifting and volcanism at about 60 Myr were followed by spreading beginning at 52–53 Myr between Greenland and Rockall. Sites 403 and 404 were drilled in a broad fault-bounded basin some 30 km east of the oldest magnetic anomaly[24] (52–53 Myr), recorded in the adjacent ocean crust. Within the basin, a thick, faulted sequence of deltaic aspect is unconformably overlain by a thin unfaulted sequence (Fig. 2). Sites 405 and 406 were drilled on ocean crust south of the east–west trending transform fault controlling the south-west margin of the Rockall Plateau, and will not be discussed here. The generalised lithology and stratigraphy of the holes drilled during Leg 48 are shown in Fig. 3.

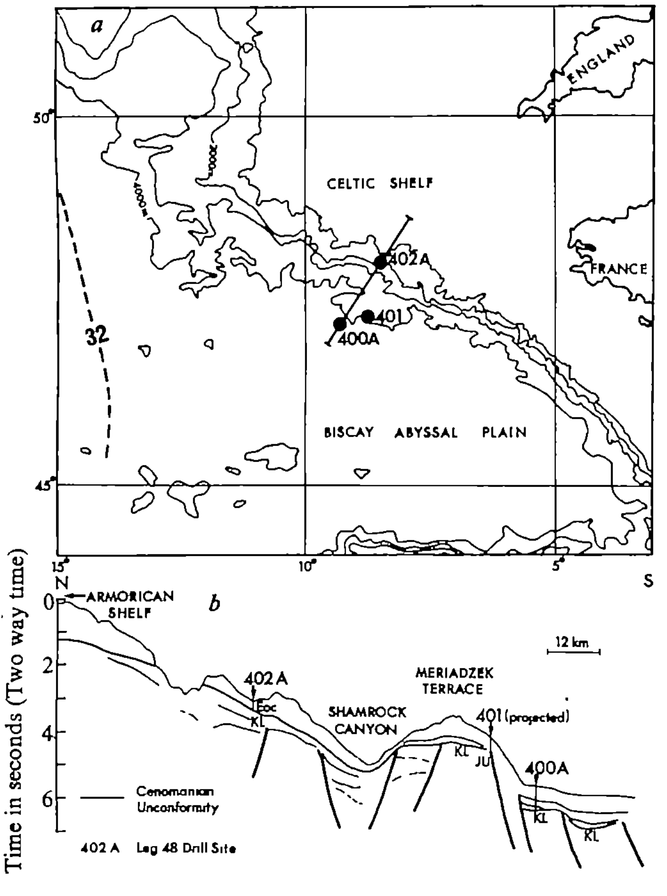


Fig. 1 *a*, Location of IPOD–DSDP Leg 48 holes in the Bay of Biscay. 32-location of magnetic anomaly-32(76 Myr). *b*, Schematic cross section of the margin. Drillsite locations shown as 402A.

Palaeobathymetry

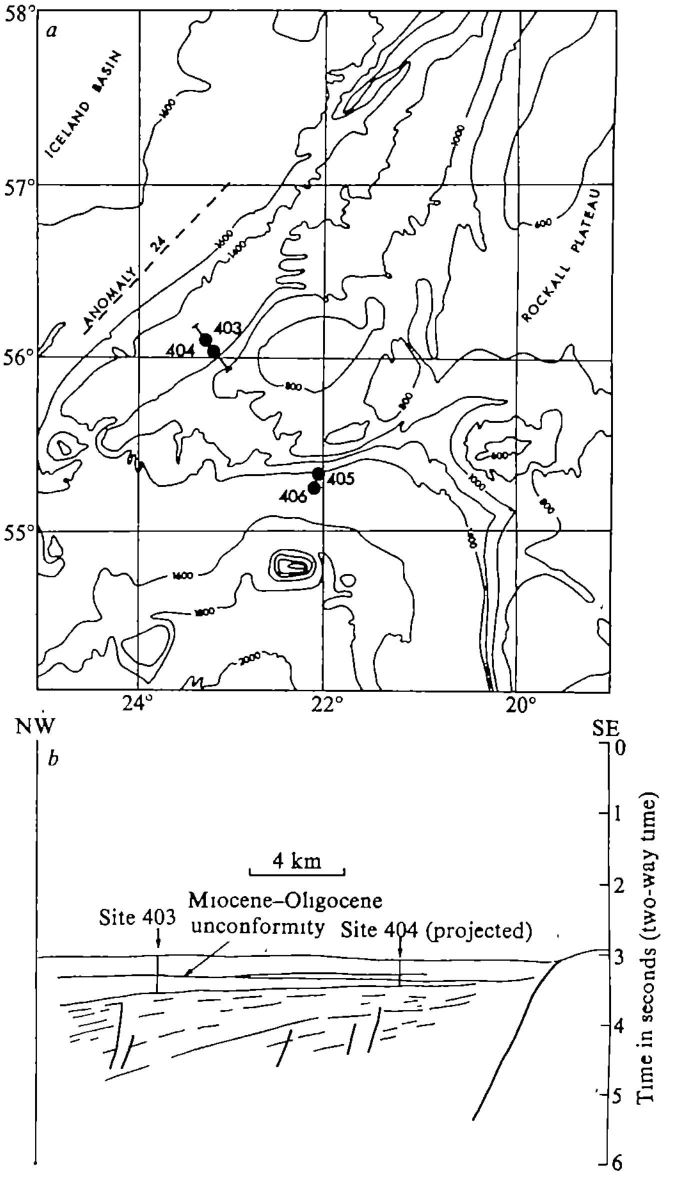
The palaeobathymetric history of the sites has been inferred principally from the macro- and microfossil assemblages, the associated lithologies, and seismic profiles which show the absence of post-rift faulting.

At site 402A (Fig. 1, Fig. 3), now situated on the upper slope of Biscay in 2,317 m depth, the Aptian carbonaceous limestones contain rare radiolaria and benthic foraminifera such as *Nodosaria* sp., *Lenticulina* sp. and *Epistomina spinulifera* indicative of a shallow shelf sea. The microfacies of the Albian limestones, as observed in thin section, are closely similar to the epicontinental Albian of the Aquitaine Basin (P. A. Du Peuble, unpublished data). In contrast, the Middle and Late Eocene nannofossil chalks contain deep-water species such as *Stilostomella* sp. However, shallow-water species, that may be displaced, are common and include *Bolivina* and *Rosalina*. The deep-water fauna suggests a depth of 1,000 to 1,500 m. The Pliocene and Pleistocene foram nanno oozes were apparently deposited at the present depth.

At site 401 (Fig 1, Fig. 3), located on the edge of the Meriadzek Terrace in 2,555 m depth, the oldest sediments encountered were Jurassic (Kimmeridgian–Portlandian) limestones containing echinoderm and pelecypod debris, algal and coral fragments, indicative of a shallow-water reefal environment. Tithonian calpionellid limestones have been dredged nearby[24]. An overlying limestone of Lower Cretaceous age (Fig. 3) contained algae, bryozoa, corals and echinoderms. Nearby dredge hauls have yielded reefal limestones of Berriasian, Neocomian and Aptian age[24]. The overlying Campanian chalk contains abundant *Stilostomella, Gyroidina, Cibicidoides*, etc., indicating a depth of deposition of at least 1,500 m, while a lower bathyl depth is indicated for the Lower and Upper Palaeocene marly nanno chalks. The Mid-Eocene assemblages suggest site 401 was then at, or close to, its present depth[25].

At site 400A (Fig. 1, Fig. 3), the Aptian planktonic faunas found in the carbonaceous marly chalks are oceanic

Fig. 2 *a*, Location of IPOD–DSDP Leg 48 holes on the south-west Rockall Plateau. *b*, Tracing of multichannel seismic section through sites 403 and 404.

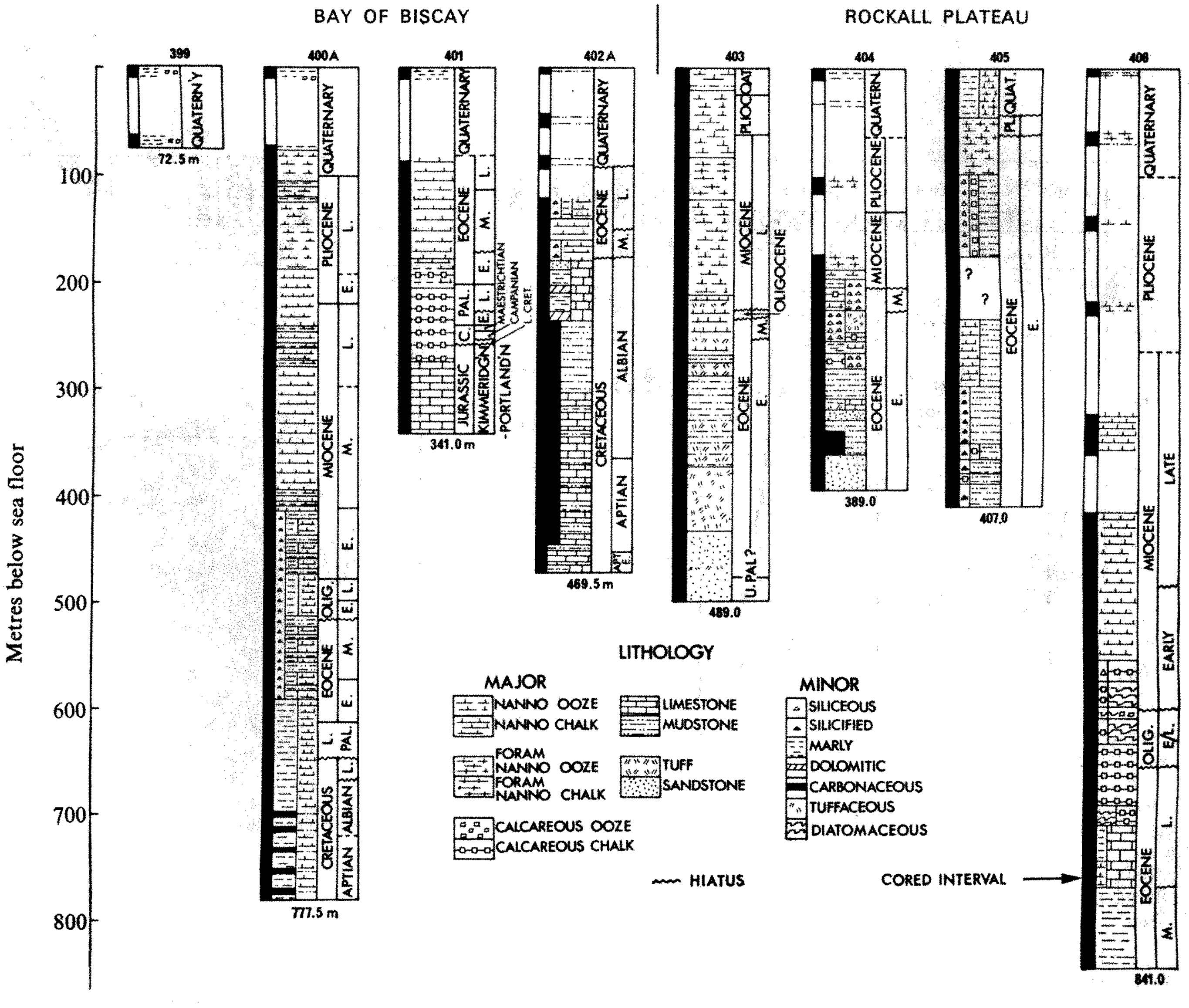


and show evidence of dissolution. The Aptian benthic foraminifera include deep-water species such as *Pleurostomella subnodosa, Gyrodinioides primitiva, Glomospira* sp. and others. The Late Cretaceous fauna has been severely affected by dissolution, and benthic foraminifera are rare though one species, *Aragonia trinitatensis* is a known deep-water form[25]. An abyssal realm of deposition for the Palaeocene claystones and marly chalks is indicated by the benthic foraminifera *Anomalina* sp., *Gavelinella* sp. and *Glomospira gordialis*. The Eocene and Oligocene foraminiferal assemblage is nearly identical to that described from the Oceanic Formation of Barbados and considered to have been deposited in 4–5,000 m depth[26]. Although these data show a progressive increase in depth since Aptian time, quantitative depth inferences are not possible due to poor preservation and the difficulty of assigning precise depth ranges to abyssal benthic faunas. Approximate palaeodepths for the site can be derived, however, from the seismic profiles in the vicinity of sites 400A and 401. These profiles show insignificant post-Aptian faulting, thus indicating that the present 2,000 m of relief between sites 400A and 401 had been created by Aptian time. The relative elevation of the Albian beds at these sites, less a small correction for the Albian palaeodepth at site 401, gives an approximate palaeodepth of site 400A in Albian time of 2,200 m. Using the same method, the Upper Cretaceous and Tertiary palaeodepths of site 401 have been transferred to site 400A and give palaeodepths consistent with the faunal evidence of contemporaneous abyssal depths at site 400A.

The 'Cenomanian' hiatus found at site 400A does not indicate subaerial erosion and uplift, for deep-water Campanian chalks rest directly on deep-water carbonaceous limestones of Albian age without evidence of intra Albian–Campanian faulting (Fig. 1, Fig. 3). At sites 401 and 402A, where the same hiatus is present, subaerial erosion and uplift are again precluded by the absence of post-Albian faulting. The missing Eocene–Lower Cretaceous and Campanian–Lower Cretaceous sections at these sites were either not deposited or were removed by erosion, perhaps by more vigorous bottom currents associated with the 'Cenomanian' transgression.

At Sites 403 and 404 (Fig. 2), on the Rockall Plateau the sequence becomes increasingly clastic downsection. Site 404 bottomed in a conglomerate containing oyster shells that suggests a littoral environment (Fig. 3). The overlying Lower Eocene contains a scarce fauna similar to the Lower Eocene 'London Clay' formation deposited in shallow water. Above 352 m deep-water species were not found, but

Fig. 3 Generalised lithology and stratigraphy of sites drilled during DSDP Leg 48.



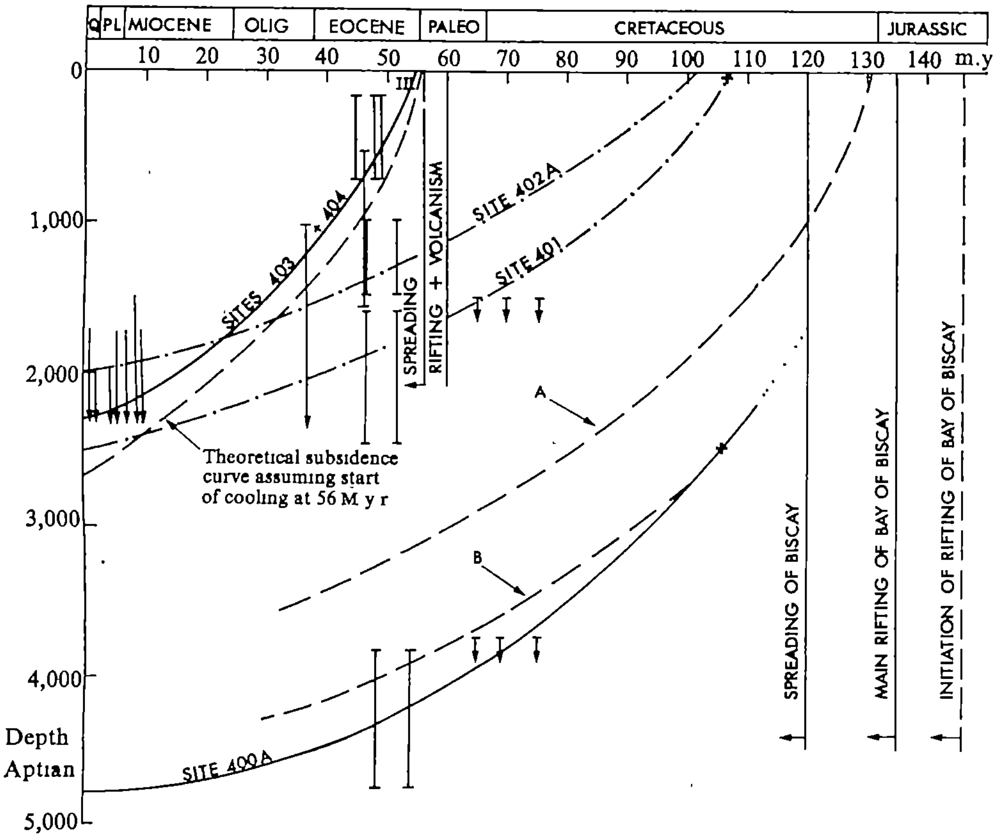


Fig. 4 Subsidence curves for sites 400A, 401, 402/402A, 403 and 404. Vertical bars indicate palaeobathymetric data derived from micropalaeontological criteria. *a*, Subsidence curve for start of cooling at 130 Myr. *b*, Fit of theoretical subsidence curve to observed subsidence curve of site 400A assuming start of cooling at 120 Myr. The curve for site 400A is based on data from sites 401 and 402A in addition. ↓ I Palaeodepth range. —·—·—, Subsidence curve sites 401 and 402A. ——, Subsidence curve sites 403 and 404.

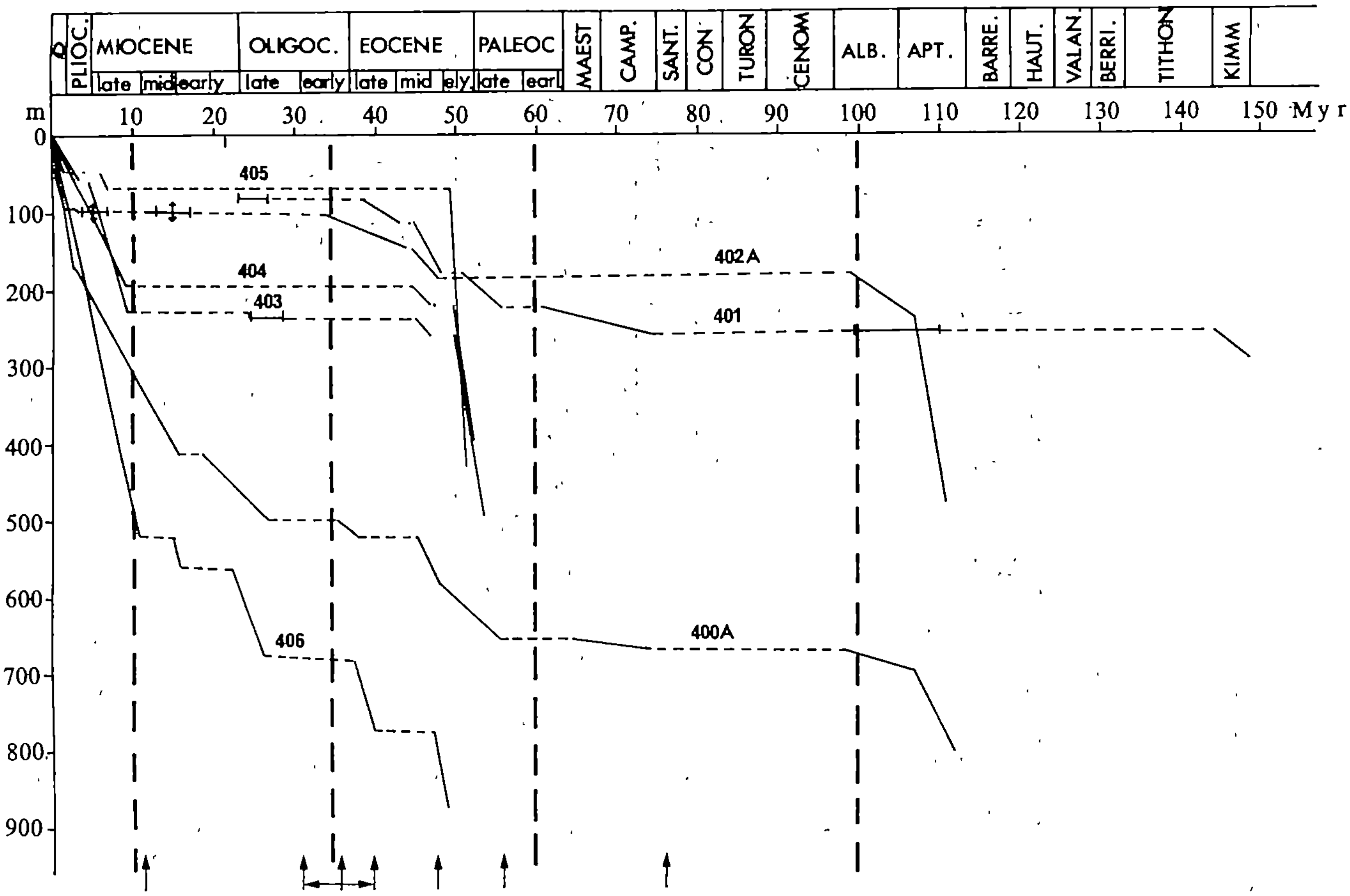


Fig. 5 Sedimentation rate curves for sites drilled in Leg 48. Changes in spreading rate and/or direction after refs 32, 33 and 34. (— — — indicates unconformites recorded on the adjacent shelf of NW Europe). ↑, Change in spreading and/or direction.

abundant plano–convex *Cibicides* indicative of shallow water are present. An upper bathyal depth during the Middle Eocene is suggested by an assemblage of bathyal and upper slope species. During the Late Miocene to the present, lower bathyal depths are indicated by the benthic foraminifera.

Rifting, spreading and subsidence

The subsidence curves given in Fig. 5 describe the depth of the sea floor at the drill sites as a function of age and are compared with theoretical age against depth curves for the Pacific Ocean crust. The curve for site 400A, however, describes the depth of the top of the Aptian as a function of age. The margin subsidence curves have not been isostatically corrected for sediment loading in view of the small sediment thicknesses at each site. In the case of site 403, the isostatically corrected depth is 2,407 m and in the case of site 400A, 4,626 m. These minor corrections further reduce the already small difference in depth between the two sets of curves.

The close similarity of the two sets of curves suggests that these passive margins have thermally subsided with a time constant that may be similar to the 50 Myr exponential time constant deduced for the ocean crust[7,27]. Although the law of subsidence thus seems to be similar, the age-versus-depth relationship for the ocean crust cannot, however, be validly extrapolated to a subsidising passive margin. This is because the absolute depth of a point on a subsiding margin depends on its initial altitude relative to sea level and not on its age, as is the case for the ocean crust. Clearly, points initially above sea level will seem to have subsided less than those below.

In understanding the application of these subsidence curves to passive margins, it is important to distinguish clearly between the thermal régimes of rifting and spreading During rifting, the continental crust is extended but remains joined so that the heat source remains fixed beneath the rift axis. In contrast, during spreading, the heat source migrates away from the passive margins as new crust accretes at the ridge axis. Cooling of the margin can only begin at the end of rifting and the onset of spreading.

If the preceeding discussion is correct, the curves can be used to infer both the onset of spreading and the palaeodepth of the margin at that time. In the case of the Rockall Plateau, extrapolation of the observed curve backward in time and comparison with the theoretical cooling curve suggests initiation of cooling at about 55 Myr. This date closely agrees with the onset of spreading at 52–53 Myr given by the age of the oldest magnetic anomaly[22] recorded in the adjacent ocean crust[21]. The curve also shows that the site was in inner shelf depths at the onset of spreading and cooling.

In the case of the Bay of Biscay, theoretical curves that assume initiation of cooling at 130 Myr for a site at sea level, lie deeper than the curves for sites 401 and 402A and shallower than that for site 400A. The closest correspondence between observed and theoretical curves for site 400A suggests initiation of cooling at about 120 Myr with the site already in 2,000 m depths. Subject to improved palaeobathymetric data, these curves suggest that spreading was initiated in 2,000 m water depths at about 120 Myr in the Bay of Biscay. This conclusion is independently supported by seismic reflection profiles which show that the Albian–Aptian carbonaceous limestones rest on the oceanic crust of the Bay of Biscay.

Although the relationship between subsidence and spreading seems the same for Biscay and Rockall, there are important and unexpected differences in the rifting and post-rifting environments. On the Rockall Plateau, and in Greenland, rifting was associated with volcanism and probably with a substantial subaerial relief, for the first post-rift sediments were deposited in inner shelf conditions at the foot of a 1,000-m scarp close to the continent–ocean boundary (Fig. 2). In Biscay, the Late Jurassic–Early Cretaceous rifting was apparently associated with minor volcanism[12,28] and produced some 2,000 m of submarine relief by the time the first post-rift sediments were laid down. We tentatively suggest that these differences may reflect rifting of an emerged Pre-Cambrian craton in the case of Rockall Plateau and, in the case of Biscay, rifting of an epicontinental Jurassic basin underlain by crust perhaps weakened during earlier Triassic rifting. The results suggest that the subsidence law for passive margins is independent of the structure and initial altitude of the continent and that passive margins begin to subside at the onset of spreading. The subsidence of both margins was accompanied by a gentle oceanward tilt of the margin rather than renewed faulting (Fig. 1, 2). However, the subsidence may have been discontinuous since hiatuses were found on the margins of Biscay and Rockall (Fig. 6). The origin and nature of such hiatuses in both the deep-sea geological record and the adjacent margins is not well understood. The reasonable correlation (Fig. 6) between the hiatuses and/or sedimentation rate changes observed on Biscay and Rockall surely implies the importance of large scale correlative events. In the deep-sea geological record, such events have usually been related to changes in ocean circulation arising from plate reorganisation and/or changes in the sill depths of the ocean basins[30]. In both Biscay and Rockall, increased sedimentation seems to follow spreading rate changes at 55 Myr and 10 Myr, though the correlation is less clear for the Oligocene–Eocene change in spreading. A more surprising correlation seems to exist between these events and unconformities recorded on the adjacent shelf and land (Fig. 6) and associated with uplift[24,31]. If this correlation is real, and validated by further deep-sea drilling, it is important and puzzling for it implies that the continental part of a plate responds vertically in coincidence with changes in spreading rate.

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Coupling of hormone receptors to adenylate cyclase of different cells by cell fusion

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A new hormone responsiveness can be conferred on an adenylate cyclase system by coupling it to a foreign hormone receptor. Receptor and enzyme from cells of different species can be compatible with each other.

In recent years, the fusion of different cells has been intensively studied[1-4]. By this process the components of the cell membrane of two different cells could be intermixed[1,5,6]. We have decided to use this phenomenon for the analysis of complex biochemical systems residing in the membrane. Thus, one could test whether a hormone receptor, or enzyme or carrier function is a dissociable unit which can couple to the homologous machinery of another type of cell. Employing the fusion process, we have recently shown that the catecholamine receptor of turkey erythrocytes is able to activiate the adenylate cyclase of mouse erythroleukaemia cells (F cells)[7].

The turkey erythrocytes were first treated with *N*-ethylmaleimide (NEM) or heat to inactivate their adenylate cyclase but not their catecholamine receptor. The F cells possessed an adenylate cyclase but no catecholamine receptor. Fusion of the two cell types with each other by Sendai virus resulted in coupling of the hormone receptor from one cell with the enzyme from the other cell. Inhibitors of protein synthesis had no effect on the process and it was, therefore, concluded that the coupling occurred between pre-existing components.

The experiment established that the catecholamine receptor is a component which is independent of the catalytic adenylate cyclase unit and that the receptor is compatible with the enzyme of another cell. However, the possibility could not be excluded that the catecholamine receptor of the erythrocyte was compatible with the enzyme of the F cell only, because both cells are of erythropoietic origin. There was also no information whether any other hormone receptor could be transferred to a heterologous system.

Present studies show that the *β*-adrenergic receptor can be donated and coupled to heterologous adenylate cyclase systems among a number of cell types of diverse origin and cellular properties. The prostaglandin E1 receptor can also be transferred to adenylate cyclase systems of other cells. Conclusions, with regard to the nature of hormone receptors which couple to adenylate cyclase, are discussed.

Transfer of the β-adrenergic receptor

The NEM-treated erythrocytes (E_{NEM}) bearing the catecholamine receptor, readily and massively fused with mouse adrenal tumour cells (Y-1) (ref. 8). Cell ghosts prepared immediately after fusion, showed a fivefold activation of adenylate cyclase by isoproterenol (Fig. 1). A control fusion of the Y-1 cells among themselves did not produce a catecholamine-responsive system. In addition, binding[18] studies showed that the Y-1 cells contained no measurable amounts of catecholamine receptor. It was concluded that activation by the catecholamine was dependent on the transfer of the receptor from the turkey erythrocytes to the Y-1 cells. In this study, basal activity of adenylate cyclase and activity in presence of fluoride were affected to some extent by the fusion process. However, this did not interfere with interpretation of the experiments since the specific hormonal response is measured as the increase over basal activity. Additional experiments showed that

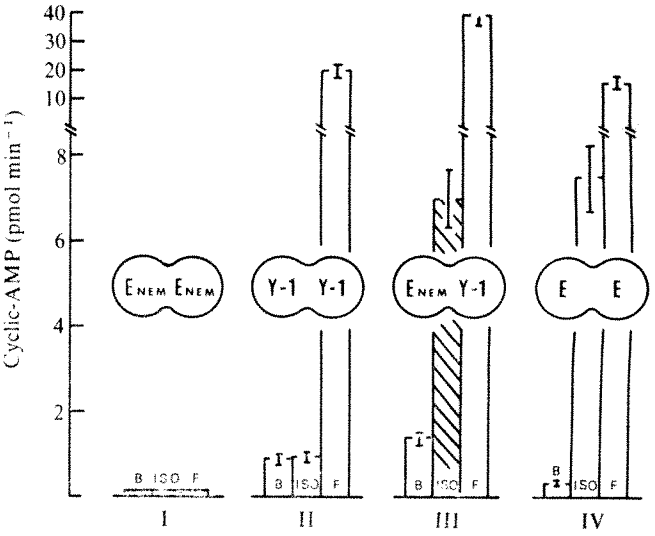

Fig. 1 Coupling of the β-adrenergic receptor of turkey erythrocytes to adenylate cyclase of rat adrenal Y-1 tumor cells by cell fusion. Adenylate cyclase activities are shown for cell ghosts prepared after fusion[7]. Systems were: (I) NEM-treated erythrocytes[7] (E_{NEM}), 3.2×10^8 cells fused with each other; (II) Y-1 cells, 3.0×10^6 fused with each other; (III) heterologous fusion of E_{NEM} cells, 3.2×10^8 with Y-1 cells, 3.0×10^6; (IV) untreated erythrocytes (E), 3.2×10^8 fused with each other. The cell types which participate in fusion are symbolised in the figure by fused circles in each set of columns. At the base of each column the activating agent is specified: B, basal activity in the presence of 20 μM propranolol; ISO, 50 μM isoproterenol; F, 10 mM fluoride. Bars at the top of the column show the range of activities of duplicate fusion systems. The response of the hormone receptor transferred by fusion to a heterologous system is emphasised by a hatched column. Adenylate cyclase activity[9] shown was measured in duplicate on cell ghosts equivalent to 1/7 of the cells in the fusion system. Y-1 cells (ATCC) were grown in Roux bottles and were subcultured as described[8]. Cells from confluent cultures (5–10 d) were suspended as follows; the monolayers of cells were washed with phosphate-buffered saline (PBS) *p*H 7.5 (140 mM NaCl, 20 mM KCl 10 mM KH_2PO_4). Two ml of PBS containing trypsin, 0.01% (w/v) (Worthington 2×crystallised and lyophylised) chymotrypsin, 0.01% (w/v) (Sigma 3×crystallised) and EDTA, 0.7 mM were placed on the cultures. After incubation for 3–5 min at 37 °C, trypsinisation was terminated by addition of 10 ml growth medium containing Soybean trypsin inhibitor (STI), 1 mg ml⁻¹. The cells were washed twice in Na^+ salt medium[7] and resuspended for fusion in Na^+ salt medium containing STI (0.5 mg ml⁻¹). Turkey erythrocytes were prepared for fusion as previously described[7]. The procedure for fusion by Sendai virus was adapted[7] from that of Loyter *et al.*[4]. Sendai virus was produced[10] by Dr Loyter of this department. The amount of virus was 3,000 HAU in systems I, III, IV and 2,000 HAU in system II. Progress of fusion was followed by phase contrast microscopy. After 45 min at 37 °C when most of the Y-1 cells were fused with erythrocytes, the cells were transferred to ice and cell ghosts were prepared by hypotonic lysis[7]. Experiments were repeated at least three times.

C6 (BU1) rat glioma cells[11,12] treated with NEM readily transferred their catecholamine receptor to F cells (Fig. 2). These findings showed that neither the erythrocytes nor the F cells used in our previous work are unique partners for the coupling experiment. A successful matching of the β-adrenergic receptor of C6 cells with the adenylate cyclase of Y-1 cells was therefore predicted and demonstrated (Fig. 3). The presence of protein synthesis inhibitors before and during Y-1 self fusion had no effect on transfer of the β-adrenergic receptor. It is, therefore, obvious that the coupling occurred between pre-existing components.

The question whether the affinity of the β-adrenergic receptor for agonists is affected by the milieu in which it operates is of considerable interest[13,14]. The ability to couple the receptor to an adenylate cyclase of another cell presented a unique opportunity to submit the problem to actual testing. It is shown in Table 1 that the dissociation constants for adrenaline, noradrenaline and propranolol do not change significantly when the β-adrenergic receptor of the erythrocytes becomes coupled to the adenylate cyclase of F cells or Y-1 cells. It is particularly noteworthy that the ratio of dissociation constants for noradrenaline–adrenaline, which serves to distinguish various types of β-adrenergic receptors[15], remains essentially unchanged.

In a further experiment rat hepatoma cells (HTC) which have no adenylate cyclase activity[16] produced a response to isoproterenol when fused with F cells. Stimulation of adenylate cyclase over basal activity was only 1.8-fold but was consistently obtained in three independent experiments (data not shown). Surprisingly, this indicates that a receptor without apparent function had been preserved throughout many months of growth of the HTC cells in suspension culture. A selected variant of HTC, grown in stationary culture, has recently been shown to bind a β-adrenergic antagonist[17].

Transfer of the prostaglandin E1 receptor

The experiments on the β-adrenergic receptor suggested that perhaps other hormone receptors could also be transferred from one adenylate cyclase system to another. Since the F cell showed strong activation of its adenylate cyclase by PGE$_1$, transfer of the PGE$_1$ receptor was attempted. Figure 4*a* shows that the adenylate cyclase system of Y-1 cells can be efficiently coupled to the PGE$_1$ receptor of NEM-treated F cells. As a control, activation of adenylate cyclase by PGE$_1$ was also tested on Y-1 cells fused with C6 cells (system III of Fig. 3 served in the test). PGE$_1$ did not increase enzyme activity of the fused preparation. Therefore, the response cannot be due to unmasking of a receptor in Y-1 by fusion *per se* with a different cell. The PGE$_1$ receptor could also be contributed by F cells which had been heat treated to inactivate the adenylate cyclase (Fig. 4*b*). Thus such experiments are not exclusively dependent on the inactivation by NEM. Fusion of the heat treated cells with each other or with HTC cells did not reactivate the adenylate cyclase. The latter is a particularly important control since HTC cells are vigorously growing though they lack adenylate cyclase activity[16]. HTC cells also did not reactivate NEM-treated F cells or NEM-treated C6 cells which were used in experiments of this study. It is also of interest that the PGE$_1$ receptor and the β-adrenergic receptor[7] are both more heat resistant than the adenylate cyclase to which

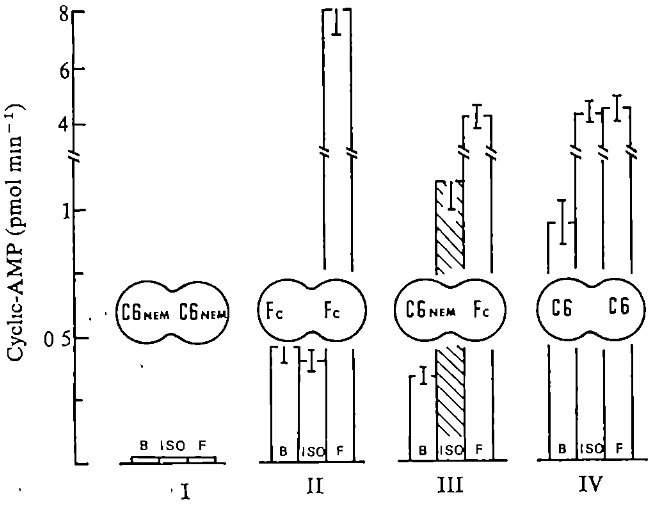

Fig. 2 Coupling of the β-adrenergic receptor of rat glioma cells (C6) with adenylate cyclase of Friend erythroleukaemia (Fc) cells. Systems were: (i) NEM-treated C6 cells ($C6_{NEM}$), 1.5×10^6, fused with each other; (ii) F cells, 1.2×10^6 fused with each other; (iii) heterologous fusion between $C6_{NEM}$ cells, 1.5×10^6 and F cells, 1.2×10^6; (iv) untreated C6 cells, 1.5×10^6, fused with each other. Other details are as in Fig. 1. F cells were grown and collected for fusion as previously described[7]. C6 cells were grown and collected for fusion as described for Y-1 cells in Fig. 1. (The culture was given by Dr U. Bachrach of this university.) The amount of virus was 400 HAU in systems i, iii and 200 HAU in ii and iv. After 22 min at 37 °C the systems were transferred to the cold and cell ghosts were prepared by hypotonic lysis.

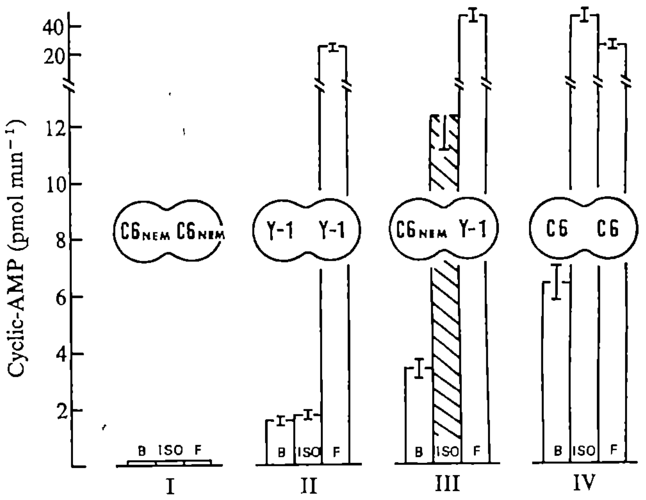

Fig. 3 Coupling of the β-adrenergic receptor of C6 cells with adenylate cyclase of Y-1 cells. Systems were: (i) $C6_{NEM}$, 1.2×10^7 fused with each other; (ii) Y-1 cells, 2.7×10^7 fused with each other; (iii) heterologous fusion between $C6_{NEM}$ cells, 1.2×10^7 and Y-1 cells, 2.7×10^6; (iv) untreated C6 cells, 1.2×10^7 fused with each other. The amount of virus was 400 HAU in systems i, ii, iv and 80 HAU in system ii. After 28 min at 37 °C the systems were placed on ice and cell ghosts were prepared. Other details are as in Fig. 1. Essentially the same results were obtained in a different experiment carried out in the presence of protein synthesis inhibitors: 15 min before fusion all cell suspensions received emetine, 20 μM and cycloheximide, 20 μM. The inhibitors remained present during fusion.

Table 1 Conservation of affinities of the β-adrenergic receptor after transfer to a heterologous adenylate cyclase system*

Cells in fusion	Apparent dissociation constants		dl-propranolol
	l-Adrenaline μM	l-Noradrenaline μM	nM
E	2.0	1.6	11
E_{NEM}–Y-1	1.8	1.1	9
E_{NEM}–Fc	1.5	1.0	—

*Fusion systems were prepared as described in legend of Fig. 1. Adenylate cyclase activities were determined on the cell ghosts in the presence of various hormone concentrations and the constants were calculated from a Lineweaver–Burk plot. Various amounts of l-epinephrine were added to 0.1 μM propranolol to determine the dissociation constant of the blocker.

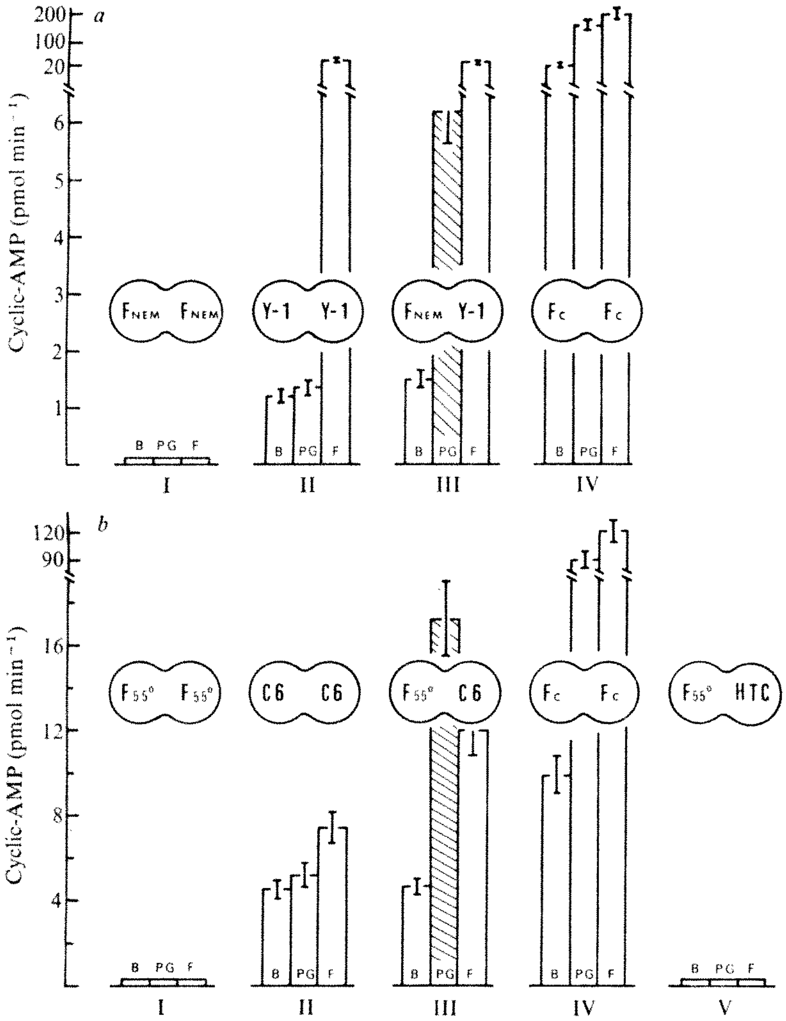

Fig. 4 Coupling of the prostaglandin E_1 receptor of F cells to adenylate cyclase of other cells. *a*, NEM-treated F cells (F_{NEM}) fused with Y-1 cells, systems were: (i) F_{NEM}, 1.3×10^7 fused with each other; (ii) Y-1 cells, 2.3×10^6 fused with each other; (iii) heterologous fusion between F_{NEM} cells, 1.3×10^7 and Y-1 cells, 2.3×10^6; (iv) untreated Fc cells, 1.3×10^7 fused with each other. Other details are as in Fig. 1, except that the hormone was prostaglandin E_1 (PG) 10 μM (given by Dr J. Pike, Upjohn Co., USA). The amount of virus was 400 HAU in systems i, iii, iv and 200 HAU in system ii. After 14 min incubation at 37 °C systems were placed on ice and cell ghosts were prepared by mild sonication[17]. Cells were sedimented by centrifugation for 3 min at 200*g* and the pellet was suspended in 0.35 ml of Na^+ salt medium containing 3 mM β-mercaptoethanol and 0.5 mg ml^{-1} STI. The cell suspension was sonicated for 15 s in a bath sonicator (Laboratory Supplies Co., Model T-80-80-1). *b*, Heat-treated F cells ($F_{55°}$) fused with C6 cells systems were: (i) $F_{55°}$ 1.2×10^7 fused with each other; (ii) C6 cells, 3×10^6 fused with each other; (iii) heterologous fusion between heated F cells, 1.2×10^7 and C6 cells, 3×10^6; (iv) F cells, 1.2×10^7 fused with each other; (v) heterologous fusion between heated F cells, 1.2×10^6 and HTC cells, 2.5×10^6. HTC cells were grown in suspension[19] and supplied by Dr R. Kulka of this department. F cells were suspended in 1.6 ml of Na^+ salt medium in a glass test tube to give 6×10^7 cells ml^{-1}. The cells were heated at 55 °C for 4 min and cooled in ice. Damage to the cells by the heat treatment was not visible by phase contrast microscopy. The amount of virus was 500 HAU in systems i, iii, iv, v and 100 HAU in system ii. After 15 min at 37 °C the fused cells were transferred to the cold and sonicated as described in Fig. 4*a*. Other details as in Fig. 1.

they were originally coupled. The PGE_1 receptor of NEM-treated F cells was also transferred to adenylate cyclase of C6 cells. PGE_1 increased enzyme activity 3.7-fold in the fused membranes (data not shown).

Experiments are in progress to transfer the ACTH receptor of the Y-1 cells to other cell types. Adenylate cyclase stimulation by ACTH in the strain of Y-1 cells used has, however, so far been rather weak and variable.

Experimental techniques

The coupling of a hormone receptor from one cell with the adenylate cyclase of another cell by the procedure described, is based on several essential requirements. Means and conditions for massive fusion must be provided because the transfer of receptor will only be evident if a considerable amount of the total adenylate cyclase in the system has been joined by the heterologous receptor. With cells having a high basal activity of adenylate cyclase, it may be difficult to demonstrate significant hormonal stimulation by coupling to a heterologous receptor. When using Sendai virus as a fusing agent the amount of virus must be accurately titrated. Both excess virus and prolonged incubation after fusion cause severe lysis of cells and inactivation of adenylate cyclase. The age and growth conditions of the cells used is also critical. For instance, adenylate cyclase in young cultures of C6 shows little response to catecholamine[20]. F cells in the stationary phase were unsuitable for heat or NEM treatment since they underwent lysis during fusion or failed to fuse. In fact, it has been our routine observation that NEM- or heat-treated cells fuse less efficiently with each other as compared with their heterologous fusion with native untreated cells. However, in turkey erythrocytes NEM treatment encouraged fusion. It is difficult to distinguish heterologous from homologous fusion when the partners are of similar appearance under the phase contrast microscope (C6, Fc and Y-1). Since NEM-treated cells fuse less readily with each other, counting the percentage of unfused cells serves as a rough indication of the extent of heterologous fusion.

Receptor–enzyme matching

It is obvious from our past[7] and present data that matching of a receptor to a heterologous adenylate cyclase system readily occurs with a considerable variety of cells of different origin, type of differentiation and method of growth. As yet, we have not encountered a fusible cell type which failed to serve in heterologous receptor–enzyme matching. Blood cells, cells grown in suspension or cells grown in monolayer and subsequently suspended by trypsinisation served equally well. The fact that the cells already possessed one native receptor (PGE_1 in F cells, ACTH in Y-1 cells, β-adrenergic in C6 cells) did not prevent acceptance of an additional transferred receptor. The same cell could contribute the adenylate cyclase, in coupling with a foreign receptor, or contribute the receptor in coupling with a foreign adenylate cyclase ($Fc–C6_{NEM}$, $F_{NEM}–C6$). These observations considerably broaden our recent conclusion[7] that the receptor is a unit independent of the enzyme and allows some suggestions as to the nature of the different hormone receptors. The unhindered transfer of two different receptors to adenylate cyclase systems of several cells suggests that the mechanism of coupling between receptors and adenylate cyclase may be universal for all eukaryotic cells. The change that must occur in the receptor upon binding of the specific hormone, which results in activation of the enzyme, is probably also identical for all these hormone receptors. Thus, it is likely that all hormone receptors which couple to adenylate cyclase have a common chemical structure, differing only in the confined area which binds the specific hormone. Because of these considerations it is expected that when hormone receptors will become available as purified proteins, it will be possible to implant them in the adenylate cyclase systems of many eukaryotic cells.

More immediate application of the technique could be to determine why OS3 and Y-6 adrenal tumour cells fail to respond to ACTH with adenylate cyclase activation[21,22] and to test for 'silent' receptors[7] as apparently demonstrated for the β-adrenergic receptor of HTC cells. It would also be interesting to see whether the β-adrenergic receptor from a unicellular organism (such as *Tetrahymena*[23]) is compatible with the adenylate cyclase of primates.

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Self-assembly and catalytic activity of the pyruvate dehydrogenase multienzyme complex of *Escherichia coli*

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Studies of the self-assembly of pyruvate dehydrogenase of Escherichia coli *show that the catalytic activity is proportional to the state of assembly and uncover a novel functional connection of active sites in this multienzyme complex.*

THE pyruvate dehydrogenase multienzyme complex of *Escherichia coli*[1,2] catalyses the overall reaction

$$\text{Pyruvate} + \text{NAD}^+ + \text{CoA} \rightarrow \text{Acetyl-CoA} + \text{NADH} + \text{H}^+ + \text{CO}_2$$

The complex, which is somewhat larger than a ribosome, is composed of multiple copies of three different types of polypeptide chain, responsible for the three constituent enzymic activities: pyruvate decarboxylase (E1) [EC 1.2.4.1], lipoate acetyltransferase (E2) [EC 2.3.1.12] and lipoamide dehydrogenase (E3) [EC 1.6.4.3]. The commonly accepted mechanism is outlined in Fig. 1.

The complex can be taken apart and the component enzymes reassembled *in vitro* to form the original structure[3] and since enzymes E1 and E3 do not associate with each other in the absence of enzyme E2, it is clear that the latter forms the structural core of the assembled complex. It is probable that enzyme E2, like the corresponding lipoate succinyltransferase from the 2-oxoglutarate dehydrogenase complex, has octahedral (432) symmetry[4], which implies that it is composed of 24 polypeptide chains[2,4]. These arguments from X-ray diffraction and electron microscopy[2,5] apparently dispose of an alternative model in which E2 comprises 16 polypeptide chains[6]. On the other hand, two conflicting versions of the number of polypeptide chains in the intact complex are current. It has been proposed[7,8] that there are about 24 chains of E1, 24 chains of E2 and about 12 chains of E3 whereas other measurements[6,9] indicate that the complex generally carries a molar excess of E1 chains, the polypeptide chain stoichiometry (E1 : E2 : E3) having an upper limit of 2 : 1 : 1 (refs 9, 10).

Three active sites in three different component enzymes participate in the overall reaction (Fig. 1). The substrate is borne by a lipoic acid residue which is covalently linked through its carboxyl group to the N^6-amino group of a lysine residue in each chain of the E2 component[11]. The lipoyl-lysine 'swinging-arm' is postulated to rotate among the catalytic centres of the three enzymes[3,12] and a study of the electron spin resonance spectra of spin-labelled lipoamide residues has provided the first convincing physicochemical evidence consistent with this hypothesis[13,27]. But, modifications to the simple reaction mechanism presented in Fig. 1 must be envisaged since we now know that each E2 chain bears not one but two functionally active lipoyl residues[14].

In this article we describe two new sets of experiments. We examine the self-assembly of the enzyme complex from the separated E1 component and E2-E3 subcomplex. We show that the chain ratio E1 : E2 reaches a limiting value of 2 : 1 and that catalytic activity in the overall reaction is proportional to the state of assembly of the complex. We go on to examine the

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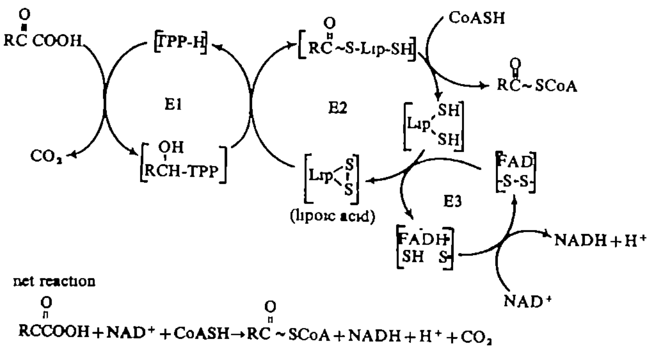

Fig. 1 The reaction mechanism of the pyruvate dehydrogenase multienzyme complex of *E. coli*. E1 (pyruvate decarboxylase, EC 1.2.4.1), E2 (lipoate acetyltransferase, EC 2.3.1.12) and E3 (lipoamide dehydrogenase, EC 1.6.4.3) represent the three enzymic activities that make up the complex. TPP-H, thiamin pyrophosphate.

fate of substrate within the complex and demonstrate participation of the lipoyl-lysine swinging-arms in subunit interactions of a novel and hitherto unsuspected kind.

Self-assembly and catalytic activity

Pyruvate dehydrogenase complex was prepared by the method of Reed and Mukherjee[15] from a strain of *E. coli* K12 constitutive for the enzyme (provided by Professor H. L. Kornberg). The complex was dissociated into free E1 and E2–E3 subcomplex at *p*H 10 and these were resolved by gel filtration on a column of Sepharose 6B (refs 16, 17). The concentrations of the resultant stock solutions were determined by amino acid analysis[18]. The polypeptide chain stoichiometry (E1: E2: E3) of the E2–E3 subcomplex was found to be 0.06: 1.0: 0.88 by means of the radio-amidination method of Bates *et al.*[9]. The E1 component gave the single band expected on sodium dodecylsulphate (SDS)–polyacrylamide gel electrophoresis. A series of partly reassembled complexes was then prepared by adding increasing amounts of E1 to a constant amount (0.29 mg) of E2–E3 subcomplex in 50 mM sodium phosphate buffer, *p*H 7.0. Each mixture was held for at least 10 h at 0 °C and any unbound E1 was then separated from assembled complex by gel filtration on Sepharose 6B. Initially, all the E1 added became bound and a single high molecular weight species emerged from the Sepharose column. But, when the amount of E1 in the assembly mixture reached 0.41 mg, free E1 component began to appear in the low molecular weight region of the column eluate and thereafter any additional E1 was found in this peak. As judged by SDS–polyacrylamide gel electrophoresis and by enzyme assay[14] of the column eluates, no E3 was displaced from the complex during the assembly.

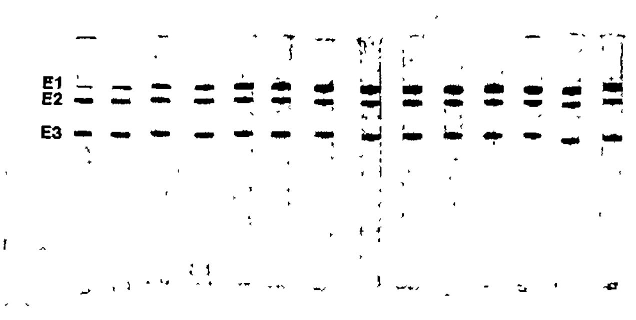

Fig. 2 Analysis by SDS–polyacrylamide gel electrophoresis of the self-assembly of the pyruvate dehydrogenase multienzyme complex. A series of partly reassembled complexes was prepared by adding increasing amounts of E1 to a constant amount (0.29 mg) of E2–E3 subcomplex in 50 mM sodium phosphate buffer, *p*H 7.0, containing 2 mM EDTA and 1 mM sodium azide. The protein concentrations were determined by amino acid analysis. After 10 h at 0 °C, the mixtures were gel filtered on a column (650 × 6.5 mm) of Sepharose 6B in the same buffer, and the fractions containing the complex were pooled. Samples were submitted to SDS–polyacrylamide gel electrophoresis in standard conditions[19] and the gels correspond, from left to right, with the points plotted in Fig. 3. The subunit molecular weights are: E1, 100,000; E2, 80,000 and E3, 56,000 (refs 6, 19).

Analysis of the series of partly reassembled complexes by SDS–polyacrylamide gel electrophoresis is shown in Fig. 2. An upper limit to the binding of E1 was quite clear, without apparent displacement of E3. A sample (about 0.25 mg) of each complex was also analysed by the radio-amidination method[9] to determine the polypeptide chain stoichiometries, which are presented in Fig. 3. These chain ratios are plotted against the molar ratio of E1 : E2 chains in the assembly mixture before gel filtration. The binding of E1 to the E2–E3 subcomplex is evidently very tight. The subcomplex is apparently saturated when 1.8 chains of E1 are bound per chain of E2, as measured by the amidination assay of the assembled complexes. This result is supported by the observation (Fig. 3) that complete assembly and activity were only reached when the molar ratio of E1 : E2 chains (the latter as subcomplex) in the assembly mixture was 2 : 1, as measured by amino acid analysis. We estimate that the binding constant for E1 to the E2–E3 subcomplex must be in excess of 10^7 M^{-1} to account for the shape of the curve in Fig. 3. The E3 content of the series was constant at a mean value of 0.85 chains per chain of E2, which does not differ significantly from the value of 0.88 in the starting subcomplex. This experiment fully supports our suggestions that the limiting polypeptide chain stoichiometry is 2 : 1 : 1 (refs 9, 10).

The E3 activity of the complex (that is, the reduction of NAD^+ by exogenous dihydrolipoamide) is unaffected by the binding of E1, as was shown by assaying a sample of E2–E3 subcomplex for this activity in the presence of increasing amounts of E1. Since the E2: E3 chain ratio for the series of reassembled complexes was constant, we could therefore use the E3 activity as a measure of the amount of subcomplex in each species. Thus we could determine how the overall pyruvate dehydrogenase activity varied with the E1 content of the complex. Accordingly, the overall complex and E3 activities of the partly assembled enzyme complexes were measured using standard assays[14] and the ratios of these activities are also plotted in Fig. 3. The increase in overall complex activity closely followed the binding of E1, maximum activity being reached only when the subcomplex had bound its full complement of E1 chains. The effective specific activity of the E1 component in the complex was therefore at least approximately constant.

Reaction mechanism and the interaction of active sites

We had previously shown that each E2 chain bears two functionally active lipoic acid residues[14]. We have now demonstrated that the maximal overall complex activity is achieved when the limiting chain stoichiometry (E1 : E2) of 2 : 1 is reached (Fig. 3). The free E1 component is thought to be a dimer[7,20] although it seems to dissociate at high *p*H into monomers[10,21]. Only the dimer is catalytically active[21]. The simplest formulation of the generally accepted mechanism (Fig. 1) would envisage direct transfer of substrate through a fixed geometric arrangement of three active sites. This proposition was tested as follows.

In the presence of the substrate 2-^{14}C-pyruvate, and the absence of CoA, the lipoic acid residues of the complex become acetylated and reaction then ceases (Fig. 1). The extent of acetylation can be measured by counting the radioactivity in samples of the complex after precipitation with trichloroacetic acid and this can then be correlated with the E1 content of the complex. A new series of partly reassembled complexes was therefore prepared as described above, except that the proportions of E1 and E2–E3 subcomplex in the assembly mixture were chosen to yield some complexes with lower E1 : E2 ratios. The ratios were then measured by the radio-amidination method[9]. Samples (each containing about 15 µg of E2–E3 subcomplex) of the partly reassembled complexes were incubated at *p*H 7.0 with 0.2 mM 2-^{14}C-pyruvate in the absence of CoA and, after 2 min, the protein was precipitated with trichloroacetic acid and counted for radioactivity. The radioactivity incorporated was plotted against the E1 : E2 chain ratio (Fig. 4): as in the previous experiment, the measured E2 : E3 chain ratio and the E3 enzymic activity were used to normalise the results to the same constant amount of E2. The maximum extent of acetylation was calculated from the specific radioactivity of the 2-^{14}C-pyruvate to be approximately two acetyl groups bound per E2 chain, in good agreement with our previous measurements of the lipoic acid content of the complex by other means[14]. It is clear from Fig. 4 that the extent of acetylation of lipoic acid residues on the E2 chains rose initially much more steeply than the E1 : E2 chain ratios of the complexes, implying that more than one E2 chain was 'serviced' by a given E1-dimer in the complex. A simple 1 : 1 correspondence of E1 chains bound with lipoic acid residues acetylated is obviously excluded.

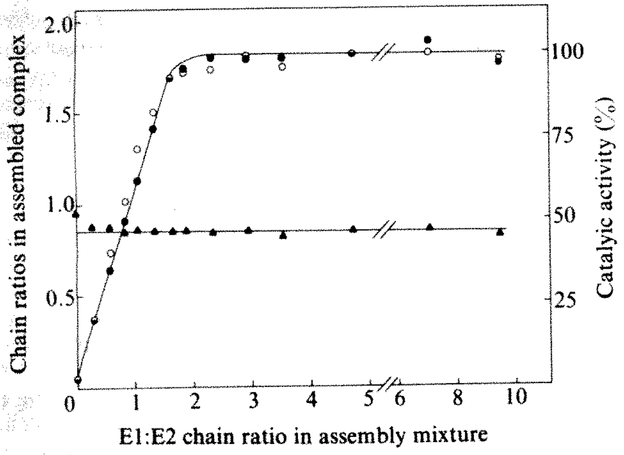

Fig. 3 The polypeptide chain stoichiometries and catalytic activities of the series of partly reassembled complexes described in Fig. 2. The polypeptide chain ratios, measured by radio-amidination[9], are based on E2 as unity. These ratios (E1, ●; E3, ▲) are plotted against the chain ratios of E1:E2 (the latter as subcomplex) in the assembly mixtures before gel filtration, as determined by amino acid analysis of the stock solutions. Five SDS gels were run for each sample and the maximum standard deviation encountered was 6%. A least-squares analysis of the first seven points gave a gradient of 0.99 and we estimate a minimum equilibrium constant of 10^7 M^{-1} for the binding of E1 to the E2–E3 subcomplex. The ratio of the overall complex activity[14] to that of the E3 component[14] for each sample is also plotted (○). The final value reached was 0.66 (compared with 0.7 in the complex before resolution) and the maximum overall complex activity recovered was 25 IU per mg, the same as the complex before resolution.

The binding of E1 to the E2–E3 subcomplex is non-cooperative and the binding sites can be assumed to be identical (ref. 10 and unpublished work of W. Busch and U. Henning, quoted in ref. 25). Let us suppose that n E2-chains can be serviced by a single E1-dimer and conversely, therefore, that a given E2-chain can be acetylated by the action of any of n different E1-dimers. Let p be the ratio of E1 dimers to E2 polypeptide chains in a given complex. Then the probability that an E1 binding site is occupied is also p. If there is one binding site for an E1-dimer on each E2 chain, which is implied by the maximum polypeptide stoichiometry of 2 : 1, then the probability that an E2 chain will not be acetylated is the same as the probability that n E1-dimers will not be bound, which is $(1-p)^n$. Thus the probability, f, that an E2 chain will be acetylated is

$$f = 1-(1-p)^n \qquad (1)$$

In the experiment, we have measured f as a function of the E1 : E2 polypeptide chain ratio, r. But $r = 2p$, so

$$f = 1-(1-r/2)^n \qquad (2)$$

This equation describes the curves which have been plotted for various integral values of n in Fig. 4. The experimental points lie closest to the line described by $n = 12$.

Rearrangement of equation (2) gives

$$\log(1-f) = n \log(1-r/2) \qquad (3)$$

Replotting the experimental data from Fig. 4 in this form gives a good straight line of gradient, $n = 12 \pm 1$.

It should be noted that the form of the curves in Fig. 4 does not depend on the assumption that E1 is active only as dimers. Identical theoretical lines are obtained if random binding of active monomers to two sites on E2 is taking place. The value of n corresponding to any curve would then be halved since it would refer to the number of E2 chains whose acetylation is caused by a monomer of E1.

In the 'servicing' experiments we have described above, the enzyme complexes were incubated with 2-^{14}C-pyruvate for 2 min before being precipitated with trichloroacetic acid. But, the same extent of acetylation was observed when the incubation time was reduced to 20 s.

Symmetry and transacetylation within the complex

The most plausible model for the structure of the E2 core of the multienzyme complex takes the form of a cube with

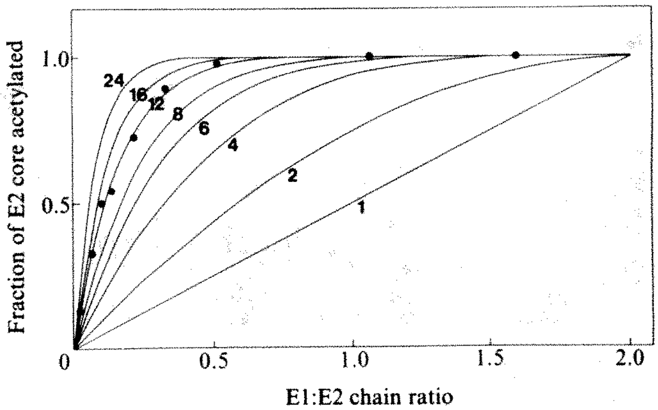

Fig. 4 Acetylation with 2-^{14}C-pyruvate of the E2 component of a series of partly reassembled pyruvate dehydrogenase multienzyme complexes. Samples of pyruvate dehydrogenase complex were incubated at 0 °C with 0.2 mM 2-^{14}C-pyruvate in 0.1 ml of 50 mM sodium phosphate buffer, *p*H 7.0, containing 0.7 mM thiamin pyrophosphate, 10 mM $MgCl_2$ and 1 mM NAD^+. After 2 min, 50 µl of rabbit immunoglobulin G (Mann Research: 20 mg ml^{-1} in 20 mM sodium phosphate buffer, *p*H 7.0) was added as carrier protein, followed immediately by 1 ml of ice-cold 12.5% trichloroacetic acid. The precipitated proteins were collected on glass microfibre filters (Whatman GF/C) and were washed with 25 ml of 12.5% trichloroacetic acid, 6 ml of acetone containing 50 mM HCl and finally 6 ml of diethyl ether. The dry filters were placed in vials with 4 ml of toluene containing 2,5-diphenyloxazole (5 gl^{-1}) and counted for radioactivity in a Nuclear Chicago Unilux II-A scintillation counter[22]. The stoichiometries of the polypeptide chains in the pyruvate dehydrogenase complexes were determined by radio-amidination[9]. The theoretical curves are plotted from the equation $f = 1-(1-r/2)^n$, for integral values of n as shown, where f = fraction of the E2 core acetylated, r = chain ratio of E1:E2 and n = number of E2 chains acetylated by the action of one E1-dimer.

octahedral symmetry, comprising 24 polypeptide chains[2,4]. It is clear from our experiments that *n* does not have the minimum value of 1 nor the potential maximum value of 24. One E1 dimer is not able to cause the acetylation of the whole E2 core. It would be expected *a priori*, however, that the observed value of *n* would be a multiple of one or more of the symmetry elements. For the octahedral model, *n* could have the values 1, 2, 3, 4, 6, 8, 12 or 24. Our present value of 12 can therefore be reconciled in principle with octahedral symmetry.

The possibility that one E1 dimer can interact direct with all 12 susceptible E2 chains simultaneously is discounted in view of the very large distances that would have to be spanned between the active sites (the complex is at least 30 nm in diameter[1,2,23]), compared with the length (1.4 nm) of the lipoyl-lysine swinging arm. Similarly, rapid migration of the E1 dimer from one binding site to another can be ruled out since an answer of $n = 24$ would be expected if the binding sites remained equivalent. We are left with the possibility that acetyl groups can be transferred between lipoyl residues bound to neighbouring E2 chains in the core, a reaction we shall term 'transacetylation' and which should be distinguished from the transfer of acetyl groups to CoA to form product (Fig. 1). The diameter of a globular E2 polypeptide chain (molecular weight 80,000; refs 19, 24) would be of the order of 6 nm, and we know that there are two lipoyl-lysine swinging arms of length 1.4 nm each per E2 chain[14], giving a potential total span of at least 5 nm for each subunit. Conformational changes in the complex might also facilitate transacetylation, so this mechanism seems structurally feasible.

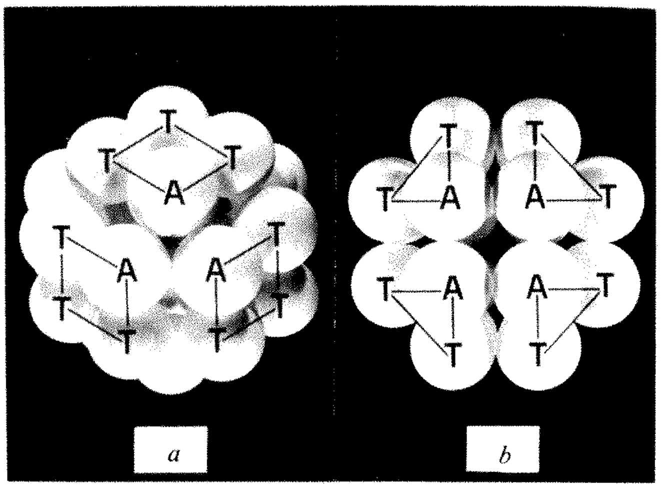

Fig. 5 Two models for the mechanism of acetylation of 12 E2 chains by means of a single E1-dimer in the pyruvate dehydrogenase multienzyme complex. The views are along (*a*), threefold and (*b*), fourfold axes of the E2 core component. The subunits acetylated by direct physical interaction with a single E1-dimer are marked with an A. Those acetylated by subsequent transacylation are marked with a T and shown linked to the appropriate subunit of type A. The total number of chains acetylated in either model is 12.

If the postulated transacetylation reactions could take place between each and every subunit in the E2 core, *n* would obviously have the value 24. To limit it to 12, we must therefore restrict the transacetylation in some way. One possible model of the E2 core with octahedral symmetry is shown in Fig. 5. Every E2 chain can be regarded as one of the three subunits in a trimer cluster at each of the eight corners of the cube or as one of the four subunits arranged about the fourfold axis on each of the six faces of the cube. We propose two possible reaction schemes. Consider a single E1 dimer bound to any one E2 chain. This E1 dimer might cause the independent acetylation of all three E2 chains in the trimer cluster at a corner of the cube by direct reaction, which would be followed by transacetylation among the four chains on each of the three cognate faces of the cube (Fig. 5*a*). Alternatively, the E1 dimer might cause the independent acetylation of four E2 chains by direct reaction with each of the subunits on a fourfold axis on the face of the cube, which would be followed by transacetylation among the three chains in the trimer clusters at each of the four corners of that face (Fig. 5*b*). We cannot for the moment choose between these two schemes but in both we must postulate that the subunits that become acetylated by direct reaction with the E1 dimer cannot engage in rapid transacetylation reactions among themselves.

Conclusions

Our results on the stoichiometry of polypeptide chains in the self-assembly of the complex substantiate our previous work[9,10] and are in good agreement with the chain ratios of the native complexes isolated by Vogel *et al.*[6] except that the highest value they recorded for the E1 : E2 chain ratio was 1.4. From our measurements of subunit stoichiometries and assuming octahedral symmetry for the E2 component, we would predict a molecular weight of about 8×10^6 for the fully assembled complex. This molecular weight predicted from symmetry arguments and protein chemistry has since been found to be in very good agreement with that measured independently by ultracentrifugation methods (M.J.D. and A. J. Rowe, unpublished).

The discovery of extensive internal transacetylation reactions in the complex has revealed a novel system of functional connection of active sites. The value of $n = 12$ gives a good fit of theory to experiment (Fig. 4) and, even if we are wrong in detail, *n* must clearly be very much greater than 1, the value implicit in the usual formulation of the mechanism (Fig. 1). Quenched-stopped-flow experiments[26] suggest that the transacetylation reactions are sufficiently fast to be kinetically competent in the normal enzymic reaction (M.J.D., A. R. Fersht and R.N.P., unpublished). They form a way of 'posting' acetyl groups and associated reduction of lipoyl residues from an E1 site to a physically distant E3 site. What advantage, if any, they confer may become apparent when the reaction is dissected in further detail.

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letters to nature

NP0532 and a hole in the Crab Nebula

THE Crab Nebula may account for as much as 35% of the dispersion measure, D, of its pulsar, NP0532 (ref. 1). Davidson and Terzian[1] have pointed out that if this is so, and if the ionised material is distributed uniformly over the volume of the nebula, then the Crab's expansion[2] of ~ 1,000 km s^{-1} should cause a decrease in D of about 0.07% per year. Isaacman and Rankin[3] studied the dispersion measure over a 5-yr period, however, and observed no such decrease. From this, an upper limit to the Crab's contribution to D can be derived. It is much smaller than previously suspected.

The variability of D is illustrated in Fig. 1; there is no secular decrease and there is a 2-yr long linear rise beginning in mid-1972. The fractional increase, to mid-1974, is about 0.1%. The times of elevated dispersion measure—from August 1969 through May 1971, and from July 1972 onward—probably occur when the line of sight is interrupted by a turbulence cell of enhanced electron density in either the Crab Nebula or the interstellar medium. The former is more likely since no other pulsars exhibit marked dispersion changes. Thus the plateau regions of D—from mid-May 1969 to mid-July 1969 and from June 1971 through August 1972—probably represent the base contribution of the nebula plus the interstellar medium The dilution of electrons in the nebula should appear as a negative slope in the plateau. Its absence allows us to place an upper limit on the Crab's contribution to D, provided that we can rule out any mechanism of increasing D that might cancel the dilution effect.

As a model, we assume that most of the contribution, D_{neb} arises from a thin spherical ionised shell of radius r and thickness Δ, rather than from a uniform sphere of electrons. We justify this on the basis of Shklovskii's observation[4] that the filaments, the highest-density regions, lie on the periphery of the nebula. (Trimble[2] has disputed this on the basis of radial velocity measurements.) Furthermore, radiation pressure will tend to sweep out the interior of a shell, at least in the beam of the pulsar.

The dispersion measure is defined as $D = \int n_e \, dl$, the electron number density integrated along the line of sight. Thus, if N electrons are uniformly distributed over the volume V of the shell

$$D_{\text{neb}} = \frac{N\Delta}{V} = N/(4\pi r^2) \tag{1}$$

Therefore the time derivative is

$$\dot{D}_{\text{neb}} = \frac{-N}{4\pi r^2} 2 \frac{\dot{r}}{r} = -2 \, D_{\text{neb}}/T \tag{2}$$

$T = r/\dot{r}$ is the 'kinetic age' of the nebula, 830 yr[2]. From the two plateaus in Fig. 1, we can place an upper limit of 5 s MHz^2 yr^{-1} on the decline of D (1 s MHz^2 = 7.43×10^{14} electrons cm^{-2} = 2.41×10^{-4} pc cm^{-3}), from which it follows that $D_{\text{neb}} < 2{,}100$ s MHz^2. This is slightly less than 1% of the total dispersion measure, 235,650 s MHz^2 (56.8 pc cm^{-3}). The result can be written as $\Delta = 0.5/n_e$ where Δ is measured in pc and n_e in cm^{-3}. For n_e comparable with filamentary densities (~10^3 cm^{-3}), the shell must be paper-thin compared with the nebula. It seems more likely that we observe the pulsar through a hole in the nebula, possibly cleaned out by the pulsar, where $n_e \sim 10$. It can now be seen that the assumption of a spherical shell was not crucial: a filled volume would require an even lower value of n_e to keep D_{neb} within the observed limit.

This determination of the Crab's fraction of the dispersion puts a contraint on the mass of ionised material it contains. For a spherical shell, the mass is

$$M = 4\pi r^2 n_e m_p (1+4f)\Delta \tag{3}$$

where m_p is the mass of the proton and f is the fractional abundance of He nuclei. Taking $f = 0.1$, $r = 1$ pc, and $n_e\Delta = D_{\text{neb}} \leq 0.5$ pc cm^{-3} gives $M = 0.21 M_{\odot}$. This must be

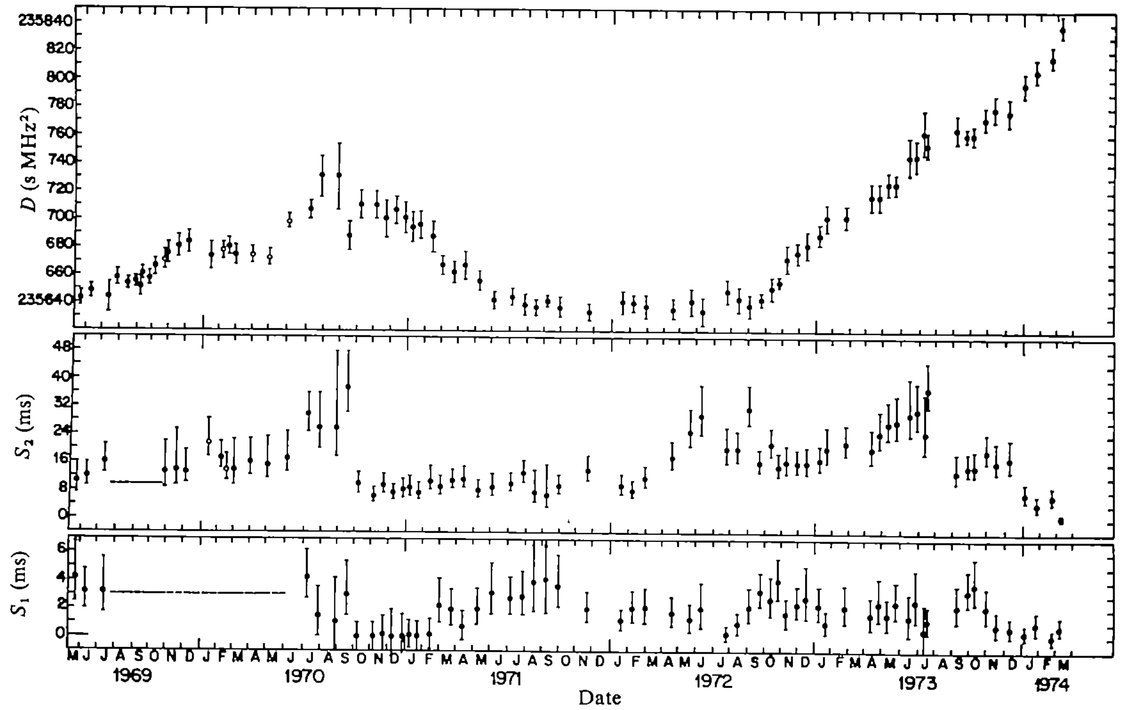

Fig. 1 Variability of the dispersion measure, D, of NP0532. (1 s MHz^2 = 7.43×10^{14} electrons cm^{-2}.) S_1 and S_2 are pulse-smearing time constants for a two-thin-screen multipath scattering model.

regarded as a lower limit, since looking through an abnormally sparse part of the nebula makes the total mass appear deceptively low. Trimble and Woltjer[5] derive $M \sim 1 M_{\odot}$ on dynamical grounds.

Since the contribution of the interstellar medium to the dispersion measure is higher than previously thought, the average electron density $\langle n_e \rangle$ in the line of sight (excluding the nebula) must be revised upwards. Davidson and Terzian derived $\langle n_e \rangle = 0.019$ cm^{-3} based on a 2-kpc distant nebula and $D_{\rm neb} = 1/3\ D$. Using a more accurate figure for the distance[2] (1.7 kpc) and $D_{\rm neb} = 0.01\ D$ gives $\langle n_e \rangle = 0.033$ cm^{-3}.

It is important that all of this holds true only if $\dot{D} = \dot{D}_{\rm neb}$, that any slope in the plateau of D in Fig. 1 is due entirely to nebular expansion. In principle, $D_{\rm neb}$ could be declining at the rate called for by Davidson and Terzian while being exactly compensated for by an increase in electron density elsewhere along the line of sight. In particular, those authors have pointed out that the Strömgren sphere engendered by ultraviolet and X-ray emission from the nebula has not had time to come to equilibrium. Integrating the Crab's spectrum[6] above the Lyman limit (by smoothly connecting the optical and X-ray spectra with a curve of spectral index 1.1) shows that the nebula puts out approximately as much ionising radiation as an 08 type star. If the density of atoms in the interstellar medium is $n_{\rm ism} = 0.15$ cm^{-3}, a Strömgren sphere of radius 70 pc will form in 5×10^5 yr. Since the nebula is less than 10^3 yr old, the ionisation front should be expanding, causing the dispersion measure of the pulsar to increase. Mitigating this effect is the extant Strömgren sphere of unknown size formed by the pre-supernova object. The contribution to D will change at the rate $\dot{D}_{\rm ss} = n_{\rm ism}\dot{R}$, where R is the velocity of the ionisation front. Provided $\dot{R} \ll 750$ km s^{-1}, we have $D_{\rm ss} \leqslant 0.5$ s MHz^2 yr^{-1}, which is insignificant. Since the expected ionisation front velocity a few tens of parsecs from the nebula is only a few hundred km s^{-1} (ref. 7), this will be the case. Hence this mechanism cannot compensate for the expansion of the nebula.

Another possible source of variation in D, which can, in principle, give rise to a systematic increase, is the 'wisps' near the pulsar, identified by Scargle[8]. These lie ~ 0.01 pc from the pulsar, and from their motions it seems that they are hydromagnetically coupled to it. However, their low surface brightness and the lack of Faraday depolarisation of the pulsar radiation implies that their density is probably very low. Since their characteristic size is ~ 0.01 pc, they contribute negligibly to the pulsar's dispersion measure.

Since the pulsar and the nebula are the only sources of ionising photons, any other mechanism for causing a secular increase in D must invoke the appropriate density fluctuations in the line of sight. The motions of these regions would have to compensate very precisely for the rate of dilution of electrons in the nebula, so their existance is improbable. We conclude that the dispersion plateau does indeed represent the base contribution of the Crab Nebula (in the line of sight to the pulsar) to the interstellar medium, from which it follows that (1) the nebula contributes less than 1% of the total dispersion measure of NP0532, (2) we are looking at the pulsar through a low-density hole in the nebula, (3) the mass of the nebula is at least $0.2 M_{\odot}$, and (4) the average electron density of the interstellar medium in the direction of the Crab is 0.033 cm^{-3}.

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The optical variability of 3C345

A SURVEY of the optical variability of compact extragalactic objects has been in progress at Asiago Observatory since 1967. Observations are taken mainly with the 67/92/215 cm Schmidt telescope on Eastman 103a-O emulsion plus a 2-mm GG 13 filter to match the B-system. A full account of the data obtained so far is now in preparation and will be published elsewhere, but it is worthwhile to report immediately on the variability of the quasar 3C345 because of its remarkable characteristics. Since Kinman *et al.*[1] discovered the large optical variations and the evidence of periodicities of 80 and 320 d, several papers have appeared reporting photometric observations of this object, whose behaviour is, therefore, unusually well covered. Also, 3C345 displays an apparent 'super-light' expansion at radio frequencies (see Cohen *et al.*[2]).

We have collected in Fig. 1, 485 B-magnitudes by Kinman *et al.*[1], Barbieri and Erculiani[3], Tritton and Selmes[4], Smyth and Wolstencroft[5], McGimsey *et al.*[6], Selmes *et al.*[7], Markova *et al.*[8], *Markova and Zhukov*[9] and from unpublished data obtained at Asiago. To simplify discussion, observations in other photometric systems (such as P-magnitudes) have been discarded and values obtained on the same day have been averaged together, so that the minimum time interval between two points in Fig. 1 is one day. The timespan covered by the data is 3,965 d. Three strong brightenings can be recognised during this period, when the object reached magnitudes brighter than the 16th. In the first and third occasion (1967 and 1971 respectively), the behaviour was very complex, with oscillations larger than 0.5 mag taking place in less than 10 d; on the second occasion (mid-1969) there was a single burst of short duration not accompanied by the slow increase seen before and after. To analyse this light curve in a quantitative way, we have employed a procedure suggested by Deeming[10] for the Fourier analysis of unequally spaced data.

A fuller discussion of our data are given elsewhere[11]; here only an important result is reported. The power spectrum PN shows three peaks, a narrow one at $\nu = 0.00065$ (period 1,540 d), a broader one at $\nu = 0.00125$ (period 800 d), and finally a smaller peak at 140 d. The region around 80 d shows an irregular structure with no definite feature, and actually the disappearance of this periodicity after a few cycles has already been discussed by Kinman[12]. The precise value of the largest period is not well determined by the timespan of the data in Fig. 1, 3,965 d, and, therefore, periods ranging from 1,420 to 1,650 d can be considered equally possible. One of them, however, is *a priori* especially attractive, that at 1,600 days, not only because it is a multiple of 800 but also because its difference of phase with the component at 800 d is almost exactly $\pi/2$ (the phases are computed from the imaginary and real parts of the Fourier transform). We have therefore attempted to synthesise the data of Fig. 1 with these three harmonics. Regarding their amplitudes, in the present situation the total lack of data when the object is close to the Sun and the scanty information available on other occasions will appreciably reduce PN at periods lower than one year. After trials, the amplitude of the component at 140 d has been increased to 0.25 mag from its computed value of 0.14 mag. The other two harmonics have amplitudes equal to $(2PN)^{1/2}$.

The solution is then

$$\begin{aligned} f(t) = {} & 16.61 + 0.35 \cos(2\pi t/1600 + 0.85) \\ & + 0.32 \cos(2\pi t/800 - 0.72) + 0.25 \cos(2\pi t/140 + 0.46) \end{aligned}$$

which is shown in Fig. 1 as the solid line. This solution, reproduces with fair accuracy the mentioned complex maxima and single burst, although it is clearly impossible to have a precise

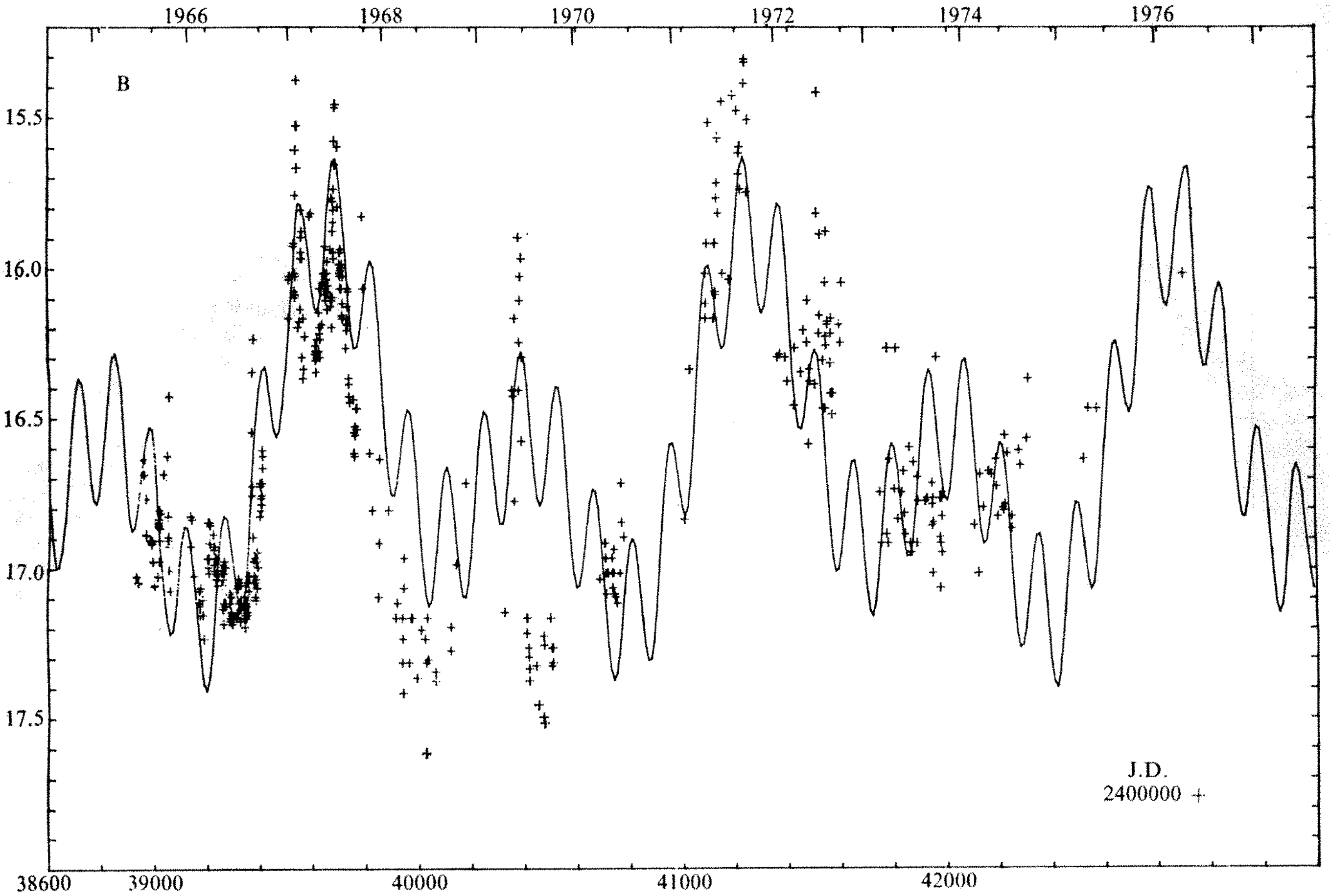

Fig. 1 The light curve of 3C345 from 1965 to 1976. +, B magnitudes taken from the literature quoted in the text. —, Harmonic solution $f(t)$ derived from our analysis.

representation of this latter feature with low-frequency components.

Other solutions with periods slightly different from 1,600 and 800 d have been explored and obviously give a rather similar representation. The best periods can be obtained only by a long extension of the data in the future or in the past. Historical data on the light curve of 3C345 have been published by Angione[13] and cover the years from 1896 to 1953. If the solution of Fig. 1 is extended over these past observations, a good agreement is found although, unfortunately, the points are too few to be conclusive evidence. Observations in the years to come will be necessary to prove beyond doubt the presence of periodic phenomena in 3C345.

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Small-amplitude, short-period waves in the E and F regions of the ionosphere

ACCURATE measurements of the virtual height of radio waves reflected from the ionosphere have been made by using a phase path ionosonde[1]. This instrument determines the virtual height, h', from the variation of echo phase with frequency, $\varphi(f)$, since $h' = (c/4\pi)(\partial\varphi/\partial f)$. Measurements are made in two ways. First, the ionosonde is swept in frequency and h' determined by taking the Fourier transform of the phase measurements on a number of frequencies. This is a form of pulse synthesis and very narrow pulses may be synthesised by combining measurements over a relatively wide frequency range[1]. The second mode of operation is to repeatedly step the ionosonde through 20 frequencies in 0.4 s so that detailed time variations and height structure can be measured. Provided there are no overlapping echoes, virtual heights can be calculated by determing $\partial\varphi/\partial f$ from adjacent frequencies. Suitable choices of frequency intervals can give very accurate values of h' (ref. 2). This paper presents results which show that small-amplitude (< 500 m peak to peak), short-period (~ 10 s) waves can occur in the E and F regions of the ionosphere.

Figure 1*a* shows an ionogram obtained by the phase path ionosonde using the pulse synthesis technique. A blanketing sporadic E layer is apparent and apart from effects due to interference at 2.5 MHz and 3.5 MHz, the virtual height is constant within 293 m over the frequency range 2–7 MHz. The value of 293 m corresponds to the quantisation level, of the pulse synthesis technique, used on this occasion. After recording this ionogram, the ionosonde was stepped from 2.0–3.9 MHz in 100-kHz steps. The variation of group path with time for several frequencies are shown in Fig. 2*a*. All frequencies show that in the 3 min for which data is presented, there is an overall

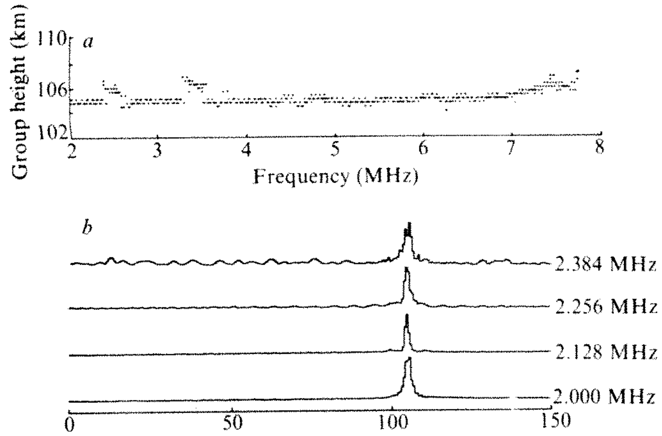

Fig. 1 *a*, An accurate ionogram produced by the phase path ionosonde using a pulse synthesis technique; *b*, examples of synthesised echoes.

rise and fall in the sporadic E layer. Superimposed on this, the three lowest frequencies show two bursts of oscillations. For the first, the period is about 11 s and the peak to peak amplitude is 240 m in virtual height. For the second burst, the period is 6.9 s and the peak-to-peak amplitude is 180 m. The oscillations are phase shifted in time on each of the frequencies and the period also varies slightly with the radio frequency. On 2.5 MHz the oscillations have decreased considerably in amplitude. Even though there is interference at 2.5 MHz, the group height can still be found since it is determined by calculating $\partial\varphi/\partial f$ using frequencies on either side of 2.5 MHz. On 3.4 MHz the first burst of oscillation is not apparent and although oscillations occur at about the time of the second burst on the lower frequencies, the period and amplitude are different. Figure 2*b* shows similar data beginning 2 min later and lasting for 180 s. During this time the behaviour is in marked contrast to that shown in Fig. 2*a*. The sporadic E layer remained at an almost constant height and although there are small variations, there are no pronounced oscillations of approximately constant period.

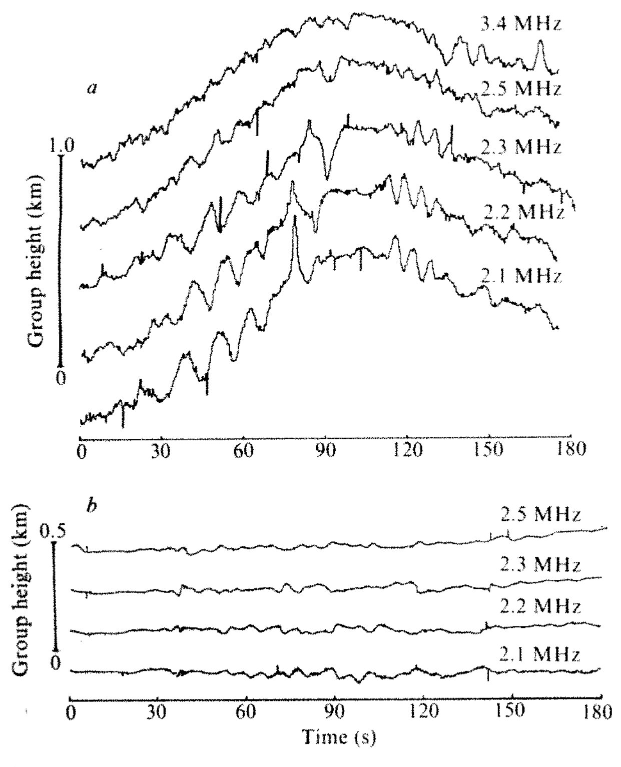

Fig. 2 *a*, The variation of group height with time on several frequencies reflected from sporadic E; *b*, similar data to *a* but beginning 2 min later.

We are confident that the bursts of oscillation represent real effects associated with the sporadic E layer. Firstly, there is strong evidence that at an instant only one reflection point is involved. The reasons for this are that the sporadic E layer has such a constant virtual height over a wide frequency range and the synthesised echoes obtained for the ionogram in Fig. 1*a* do not show pulse spreading (see Fig. 1*b*). Secondly, if they were an artefact of the instrument (such as beating with the mains supply), the oscillations would appear on all frequencies and the time variations would be in phase and have the same period. The third point to be considered is that the antenna system used was not polarised so that there is no discrimination between ordinary and extraordinary propagation modes. The constant virtual height against frequency of the sporadic E layer indicates that there is relatively little ionisation below this layer, so that the virtual range of ordinary and extraordinary rays will differ only marginally. Since the extraordinary mode is strongly absorbed, our observations basically refer to the ordinary mode. However, one question to be answered is whether the variations shown in Fig. 2*a* could be caused by differential absorption effects. If such effects are due to time variations in the D layer, and, therefore, well below the reflection level, the time variations on the different frequencies should be in phase. If such effects occur near the reflection level then it means that the small-period oscillations are in fact occuring in the sporadic E layer.

The period of the oscillations is too short to be gravity waves but they could be sound waves. However, the fact that the oscillations occur only on a few frequencies suggests that they may be confined to a small part of the sporadic E layer and since they occur for only a short time, we may be observing bursts of irregularities produced by plasma instabilities occurring in the sporadic E layer. The amplitude of the oscillations is small but cannot be directly interpreted as the changes which are occurring in the real height of the reflection point because virtual height changes can be an overestimate of the true height changes and angle of arrival effects can occur as well.

Oscillations of short period (~ 4 s) and small amplitude (~ 250 m peak to peak) have also been observed in the F region but they are not so confined in height. Again they may well be the effects of either sound waves or plasma instabilities. Such short-period small-amplitude phenomena do not seem to have been detected in the ionosphere before and they are being studied further in order to explain them in detail.

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Radioactive ^{38}S in an atmospheric SO_2 and SO_2 oxidation rate measurement

BHANDARI *et al.*[1] and independently Perkins *et al.*[2] have detected cosmic-ray-produced ^{38}S in several precipitation samples. This paper reports on the first detection of cosmic-ray-produced radioactive ^{38}S as atmospheric $^{38}SO_2$. The use of this nuclide as a tracer for the *in situ* determination of SO_2 oxidation rates is also discussed. ^{38}S is produced by spallation of atmospheric argon and decays with a half life of 2.87 h by β^--emission to ^{38}Cl; 95% of the decays are accompanied by 1.88 MeV-γ-radiation, 5% undergo direct transition to the ^{38}Cl ground state.

The present work on the *in situ* measurement of SO_2 oxidation rates within the complex system of the natural atmosphere (where no other direct method to determine SO_2 conversion rates is known) began because we felt this could be a very interesting application of cosmic-ray-produced sulphur.

The radioactive sulphur, which after the spallation is present as sulphur dioxide, participates in the chemical fate of environmental SO_2 and is oxidised and converted to sulphate in the same manner and $^{38}SO_4{}^{2-}$ also decays. The ratio of radioactive SO_2 to radioactive $SO_4{}^{2-}$ depends on the ratio of the radioactive decay rate to the SO_2 conversion rate. Thus simultaneous measurements of ^{38}S-radioactivity in SO_2 and in particulate, $SO_4{}^{2-}$ should give information on the SO_2 conversion rates.

For sampling SO_2, atmospheric air is sucked, with a flow rate of about 150 $m^3\ h^{-1}$, through a paper filter (Macherey, Nagel & Co. MN 714) for removal of particulate matter and then through an absorption chamber. In this absorption chamber, a solution of 0.1 M NaOH and 1.5% H_2O_2 is sprayed into the air stream. More than 97% of SO_2 is absorbed by the dispersed solution. Within this solution SO_2 is oxidised and is precipitated as $BaSO_4$. Sulphate is reduced to H_2S by a procedure similar to that described by Polson and Strickland[3], and by Stoeppler[4]. H_2S is directly used as counting gas in an internal-gas β-radiation detector. Details of the sampling procedure as well as of internal-gas counting will be published elsewhere.

In order to reduce detector background, and to discriminate against ^{35}S (half life 87 d; no γ-radiation) which is also present in atmospheric samples, low-level β-γ-coincidence technique as described by Roedel[5] is used. The internal-gas counter tube of ~26 cm^3 volume is fitted in the well of a 5 × 5-inch-NaJ(Tl)-scintillation crystal. The scintillation crystal registers the γ radiation and is gated by the β radiation detected in the gas counter. For the 1.88-MeV γ peak there is a detection efficiency of $7\pm0.8\%$ and a background counting rate less than 10^{-3} events min^{-1}.

Five samples have been measured between 5 February and 3 March 1976. The activities varied between 1 and 2×10^{-3} d.p.m. $^{38}S\ m^{-3}$, the mean being 1.3×10^{-3} d.p.m. m^{-3} (d.p.m. is disintegrations per minute). Sample volumes varied between 325 m^3 and 520 m^3. Due to the very poor counting statistics the uncertainties are still very great (about $\pm0.7\times10^{-3}$ d.p.m. m^{-3}).

Unfortunately, it is not yet possible to measure ^{38}S as airborne particulate $^{38}SO_4{}^{2-}$. As mentioned above a filter is used to retain and collect aerosols. This filter, however, causes also an appreciable retention of SO_2. This effect is most probably due to catalytic oxidation of SO_2 to $SO_4{}^{2-}$, induced, for the most part, by the collected aerosols. The loss of SO_2 amounts to ~10% in new unloaded filters and to ~30–50% in filters loaded with aerosols. This indicates that ^{38}S-activity found on the filters is, for the greater part, due to a contamination by SO_2 converted within the filters. An improved sampling technique, however, is being developed which, together with an increased efficiency of radiation detectors, should allow simultaneous measurements of $^{38}SO_2$ and $^{38}SO_4{}^{2-}$.

Finally, we shall give a rough estimation of the limits of the radiosulphur method. For this estimate only first-order reactions for the SO_2 conversion are allowed, further ^{38}S is assumed to be primarily present as SO_2 (this has still to be investigated), and transport and removal processes are ignored. Then the following equation for the $^{38}SO_2/^{38}SO_4{}^{2-}$ balance holds

$$d[^{38}SO_4{}^{2-}]/dt = +k[^{38}SO_2]-\lambda[^{38}SO_4{}^{2-}] \qquad (1)$$

(λ and *k* are the constants of radioactive decay and of conversion, respectively).

In a steady state, equation (1) reduces to

$$k = \lambda[^{38}SO_4{}^{2-}]/[^{38}SO_2] \qquad (2)$$

If a ratio $[^{38}SO_4{}^{2-}]/[^{38}SO_2]$ in the order of 0.1 can be detected, the minimum conversion rate which can be determined, is $k = 0.1\ \lambda$ (with $\lambda = 0.242\ h^{-1}$) or about 2.5% h^{-1}. Laboratory experiments suggest that reaction rates for homogeneous SO_2 oxidation in urban air lie between 0 and 10% h^{-1} (Cox and Penkett[6], Cox[7]). Haury and Jordan[8,9] report reaction rates for heterogeneous oxidation on the surface of aerosol particles up to 15% h^{-1} depending on the relative humidity (Haury's and Jordan's measurements, however, are made at very high concentrations of SO_2 and aerosols).

The expression for *k* given above does not depend on the ^{38}S production rate. Nevertheless, the specific activity of $^{38}SO_2$ may be affected by the vertical variance of the production rate. In the lower atmosphere, the spallation rate increases by a factor of ~2 with every 1.5 km up[10,11], a part of this elevated activity may be mixed downwards by eddy diffusion. The maximum additional increase of the $^{38}SO_2$ concentration can be estimated by Fick's equation for the turbulent mixing

$$\frac{1}{[^{38}SO_2]}\ \frac{\partial[^{38}SO_2]}{\partial t}\bigg|_{mix} = \frac{1}{[^{38}SO_2]}\ \frac{\partial}{\partial z}\left(K_z\ \frac{\partial[^{38}SO_2]}{\partial z}\right).$$

$$\approx \frac{1}{[^{38}SO_2]_{z=0}}\ K_z\ \frac{[^{38}SO_2]_{z=z_0}}{z_0{}^2} \approx 2K_z/z_0{}^2 \approx 6.5\%\ h^{-1} \qquad (3)$$

(K_z being the coefficient of vertical eddy diffusion, which is assumed to be $2\times10^5\ cm^2\ s^{-1}$, and $z_0 = 1.5$ km being the scale height for doubling the production rate, *z* is the vertical axis) or an increase of the $^{38}SO_2/^{38}SO_4{}^{2-}$-ratio of 20–25% within the lifetime of ^{38}S. This estimate, however, gives an upper limit for the error in the $^{38}SO_2/^{38}SO_4{}^{2-}$ ratio. In most cases one may assume an averaging within the scale of $\sigma_z = \surd(2K_Z/\lambda) \approx 750$ m rather than an appreciable increase of the $^{38}SO_2/^{38}SO_4{}^{2-}$ ratio.

Furthermore, the SO_2 to $SO_4{}^{2-}$ balance in the real atmosphere may be affected by horizontal ventilation and by the uptake of SO_2 by plants and soils (see, for example, ref. 12 and references quoted therein).

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Stresses at spherulite boundaries

IN a recent study of drawing behaviour in polyethylene Barham and Keller[1] discussed the black lines which are often seen around spherulites of polyethylene viewed between crossed-polarisers. They state that these dark lines seem to be independent of their orientation with respect to the polarisation directions and attribute them to poorly crystalline regions of low molecular weight material. Here I offer an explanation for these lines and show that they reveal the presence of stresses at the spherulite boundaries.

Figure 1 shows a 5-μm thick film of polyethylene ($\overline{M}_n = 11{,}100$, $\overline{M}_w = 68{,}000$) prepared by dropping a *p*-xylene solution on to a hot microscope slide, drying and melting the polymer and rapidly cooling. The dark lines surrounding the banded spherulites can be seen. Note that boundaries are not dark if they make an angle of between about 30°–60° to the polarisation directions parallel to either edge of the figure. Rotation of the sample causes the dark bands to become as light as the rest of the spherulite, when the boundary is inclined at about 45° to the polarisation directions.

This suggests that the boundaries contain oriented polymer; but similar dark lines can be seen at boundaries between small, suitably oriented non-polymeric crystals. One explanation is that the dark band arises when light crosses the boundary during its passage through the specimen. The equations for polarised light passing through two superimposed crystal plates[2] show that extinction can occur when the slow and fast optical axes have a mirror symmetry across the boundary and the boundary is parallel to one of the polarisers. If the optical axes make an angle of 45° with the boundary the intensity I is given by $I = \sin^2[(\delta_1 - \delta_2)/2]$ where δ_1 and δ_2 are the two retardations. Thus, extinction will occur if the retardations are equal. If the angle of the optical axes to the boundary varies from 45° the system will still be close to extinction. If the boundary is tilted an arbitrarily small amount with respect to the light path, slightly different light paths will cross different thicknesses of the two crystal plates, and there will be one path where extinction occurs. The argument is more involved for banded spherulites such as polyethylene, but the result is still that an extinction line should be seen at the boundary and there is no reason to postulate the presence of a boundary film to explain the black line.

Further information can be derived by considering the regions of the spherulite where the boundary line zig-zags as can be seen in Fig. 1. Corresponding zig-zags can also be seen by using light Nomarski interference microscopy and scanning electron microscopy (SEM) though these lack the clarity and contrast of the polarised light views. Three origins for these zig-zags seem possible. The first is that the boundary may be tilted so much that the extinction position wanders from side to side. This idea is eliminated by observing these uneven boundaries by SEM and reflected light, and by the lack of any significant change as the sample is tilted during polarised light observation. The second possibility is that the two spherulites interpenetrate to produce a zone of changing birefringence. The observation of similar black lines between suitably oriented crystals of simple compounds shows that interpenetration is not necessary. The SEM and reflectance microscopy confirm the third possibility, that the zig-zags are real.

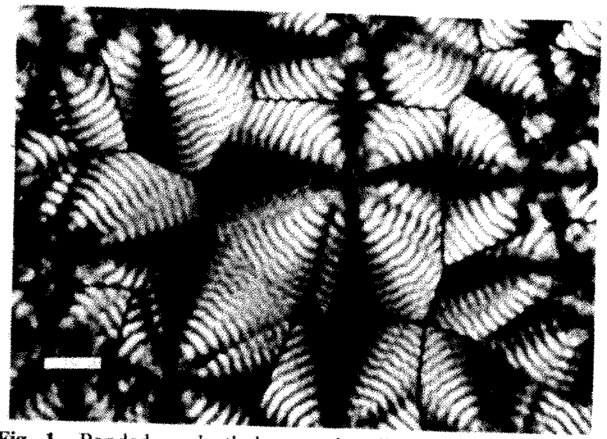

Fig. 1 Banded polyethylene spherulites showing zig-zag boundaries. Scale bar 10 μm.

These zig-zags occur where the band structures in the two spherulites intersect at an angle of about 90° and also where the banding is so out of phase that a dark band in one spherulite is crystallising adjacent to a light band in the other spherulite. In these circumstances the light band always protrudes into the dark one. Thus the light band, where the chains within the crystals are in the plane of the film and perpendicular to the spherulite radius[3], grows faster than the dark band, where the chains are perpendicular to both the plane of the film and the spherulite radius. Since the crystal twisting frequency in angle per unit length is unchanged, the net result is that each spherulite alternately cuts across the front of the other then slows down as the lamellar crystals twist into the dark orientation. This is illustrated in Fig. 2. This effect is not restricted to thin films; the same zig-zag boundaries with the same light–dark relationship are observed in some regions of microtomed slices of bulk samples.

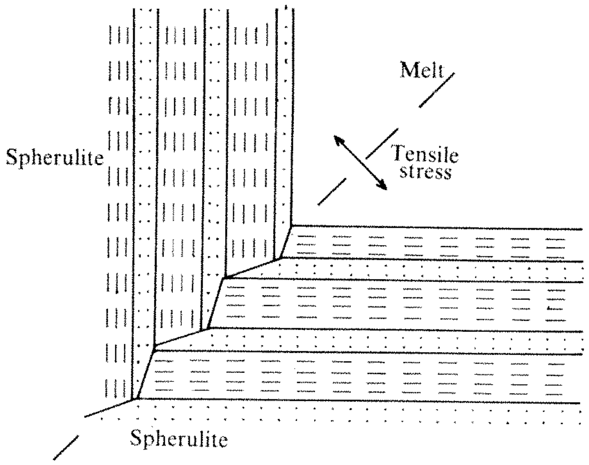

Fig. 2 Structure at a zig-zag boundary. Lined bands: bright, chains parallel to lines. Dotted bands: dark, chains perpendicular to paper.

In a bulk sample, when a microtomed section contains the centres of two spherulites and their common boundary, the plane of the boundary must be perpendicular to that of the section. The dark bands will then always contain crystals aligned with the chains parallel to the boundary and perpendicular to the section, while the chains in the bright bands are at an angle to the boundary.

The 10% shrinkage that occurs as spherulites form will produce a radial tensile stress and tangential compressive stress in the melt which will decay at a rate characterised by the melt relaxation time[4]. Where spherulites approach, the liquid layer trapped between the two may be unable to relax and a tensile stress perpendicular to the common boundary will develop.

The case where zig-zags form is shown in Fig. 2. Here the spherulite radii are both at 45° to the boundary and the banding is out of step. From studies on cross-linked polymers it is known that tensile stress increases the growth rate of crystals where the chain axis is parallel to the stress axis[5]. In Fig. 2 the tensile stress will have a component parallel to the chain axis in the light band but not in the dark band, so that the growth rate of the light band will be increased.

From the angle of the zig-zag we can make an order of magnitude estimate of the boundary stress. Taking the angle between the zig and zag to be about 60° we obtain a growth rate ratio of 1 : 1.5. Putting this into a thermodynamic theory of polymer crystal growth under stress[6], taking the undercooling as 30 °C and assuming that the stress does not affect the growth rate transverse to the stress we obtain a local strain of about 0.3 or a stress of about 6×10^5 N m^{-2} (6×10^6 dyn cm^{-2}). This can be taken as an average strain over a zone corresponding to the span of the zig-zag.

Black boundary lines having similar properties have been seen in several spherulitic polymers formed as thin films on glass slides. In some cases non-birefringent dark lines were also seen. Barham and Keller have shown that the extent to which these lines can be seen depends on the molecular weight distribution and crystallisation conditions. Polyoxymethylene and poly(ethylene adipate) form banded spherulites where the boundaries are often irregular but do not show clear zig-zags. Residual boundary stresses of this magnitude must affect the strength and oxidation and corrosion resistance of polymers.

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Transport of metal complex chlorides through plastic film membrane

THE acid–metal–chloro complexes of gallium and iron have been shown to be transported through polyurethane membranes by virtue of an acid or chloride gradient. Vofsi and co-workers[1–3] have reported on transport of uranium using complexing plasticisers in thin plastic films. More recently, the transport of sodium across a liquid membrane was described[4]. Recent work[5] has demonstrated that open cell polyurethane foam sponge with surface areas of less than 0.1 m^2 g^{-1} can dissolve gallium or iron as the HMCl$_4$ complex to as much as 10% of the foam by weight. In this note, we investigate the transport of metal complexes, such as HMX$_4$ of gallium and iron, across polyurethane plastic films. We believe that the acid-chloro complex could dissolve in the film and so pass through such membranes.

Our all-glass diffusion apparatus consisted of two equal volume flanged cells with top openings for sampling. The 125-μm polyurethane film (ether type) was sealed vertically between the two glass flanges with high vacuum Silicone or Kel-F grease and clamped. Platinum electrodes sealed in glass were used to determine the specific conductance of the water (receiver) side which indicated the extent of migration of gallium from the loaded cell. The apparatus was immersed in a constant temperature bath controlled at 20±1 °C and the solutions were stirred with magnetic stirrer and Teflon-coated magnets. Small (1 or 2 ml) samples were periodically removed from the receiver side (Fig. 1) or both sides (Fig. 2) of the diffusion apparatus and analysed for acid and metal. Gallium was determined by AAS (ref. 6), whereas iron and uranium were analysed spectrophotometrically by the thiocyanate complex.

The effect of acid strength and chloride concentration on the extent of diffusion of gallium through the polyurethane membrane is shown in Fig. 1. The results for the diffusion of 200 p.p.m. gallium from 3.10 M HCl is not shown but after 500 h gave less than 1 p.p.m. gallium in the water cell which had a specific conductance of 0.17×10^{-3} Ω^{-1} cm^{-1}. The rate of diffusion of gallium is qualitatively similar to the solvent extraction efficiency of the polyurethane foam[5,7], and is believed to be due to the equilibria

$$\mathrm{H^+ + Ga^{3+} + 4\,Cl^- \rightleftharpoons HGaCl_4(aq)} \qquad (1)$$

$$\mathrm{HGaCl_4(aq) \rightleftharpoons HGaCl_4(foam)} \qquad (2)$$

The depletion of gallium from the loaded cells was determined analytically and Ga was found to have decreased from 200 p.p.m. initially to less than 1 p.p.m. at the conclusion of the experiments. The final concentration of gallium in the receiver cell was, in some cases, greater than the initial 200 p.p.m. (the membrane initially separated equal volumes of solution) because of an osmotic effect which was noticed when most of the gallium had diffused through the membrane. The osmotic effect depleted water from the water cell causing the volume of the loaded cell to increase. The volume of water transported was about 10–20% of the total volume. This was primarily accommodated by the bulging of the membrane into the water cell.

The mechanism of transport is readily explained by equations (1) and (2). The HGaCl$_4$ complex is formed in the acid (loaded) cell dissolves in the membrane through which it can diffuse. As the complex appears on the water (receiver) side it dissociates back to the component ions, H$^+$, Ga^{3+} and Cl$^-$. The Ga^{3+} ion cannot diffuse back until the concentration of the HCl has increased significantly to reform the HGaCl$_4$ complex. This back diffusion has been observed with iron (III) where it was also noted that the initial hydrolysis of the HFeCl$_4$ complex in

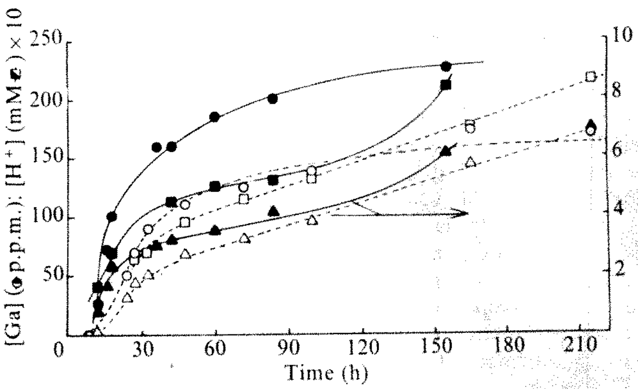

Fig. 1 Time dependence of specific conductance (κ, △▲) and diffusion of gallium (○●) and acid (□■) through 9.1-cm^2 polyurethane membranes into 130 ml distilled water. Initial conditions: *a*, solid symbols, 200 p.p.m. Ga, 6.07 M HCl, 130 ml. *b*, Open symbols, 200 p.p.m. Ga, 3.12 M HCl, 3.00 M LiCl, 130 ml.

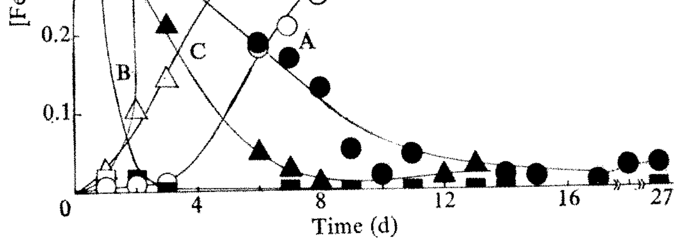

Fig. 2 Diffusion of iron from loaded cell (solid symbols) through polyurethane membranes into receiver cell (open symbols) as a function of time. *a*, Circles: 620 ml of 0.4 mg ml^{-1} Fe^{3+}, 2 M HCl, 5 M LiCl with 620 ml of 2 M HCl in receiver cell, membrane area 42 cm^2. *b*, Squares: 300 ml of 0.4 mg ml^{-1} Fe^{3+}, 2 M HCl, 5 M LiCl with 300 ml of 0.1 M HCl in receiver cell, membrane area 21 cm^2. *c*, Triangles: 750 ml of 0.4 mg ml^{-1} Fe^{3+}, 2 M HCl, 5 M LiCl, with 750 ml of 2 M HCl in receiver cell, membrane area 50 cm^2. Cell preconditioned with HCl and LiCl for 6 d before Fe^{3+} was added.

water precipitated the red-brown $Fe(OH)_3$ which was deposited on the water side of the membrane. The precipitate then dissolved slowly as the acid diffused through the membrane. This may have occurred for gallium but was not noticed because the $Ga(OH)_3$ is not coloured. The formation of the $Fe(OH)_3$ indicates that the acid–metal complex diffuses through the membrane faster than HCl.

When a low-acid (3 M HCl) gallium system was studied with added LiCl (3 M) the diffusion rate was much faster than for 3 M HCl but not as fast as for 6 M HCl. This is consistent with the formation of the complex and its solubility in polyurethane[5]. On completion of the experiment (230 h) (Fig. 1 *b*), analysis for lithium by AAS in the water cell showed that less than 1 p.p.m. may have diffused through the membrane.

The results for the diffusion of iron (III) through the membrane are shown in Fig. 2, where the receiver cell contained 2 M and 0.1 M HCl to prevent the hydrolysis of the iron complex to insoluble hydroxide. The rate of diffusion of the iron is greatly affected by the strength of the acid in the receiver cell. The time lag noted for the appearance of metal in the receiver cell was significantly reduced when the membrane was conditioned for six days in the cell with acid and LiCl before the iron was added (Fig. 2 *c*). This implies that the mechanism of transport of the metal is primarily via the anion complex, MCl_4^-, which jumps from one protonated ether group to another. The membrane morphology had changed considerably during the diffusion experiments and the appearance of blisters, vacuoles, resemble the changes observed by Vofsi[8] with plasticised membranes.

The diffusion of iron (III) chloride in 6 M HCl solution was tested with membranes of 50 μm polyethylene, 25 μm Saran, 25 μm PVC and 50 μm Teflon. In all these cases no detectable iron had diffused through the membrane in 15 days. Iron (II) chloride in 6 M HCl did not diffuse through the polyurethane membrane whereas iron (III) bromide in HBr solution was slightly slower than the iron (III) chloride. Preliminary experiments with $UO_2(NO_3)_2$ in the presence of $Al(NO_3)_3$ showed diffusion of uranium to be occurring through polyurethane membrane.

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Long-period effects in the Romanian earthquake of March 1977

THE field study of the Romanian earthquake of 1977 suggests that strong ground motions, for engineering purposes, may differ considerably from those currently adopted for design on the basis of US West Coast-type of recordings. The earthquake of March 4 1977 in Romania had a magnitude of $M = 7.2$ and a depth of about 90 km. It originated from the source of intermediate-depth earthquakes of Vrancea, beneath the bend of the Carpathian mountain arc, at 45.8°N, 26.7°E, a source similar to that of intermediate events in the Hindu Kush.

It was felt over an area of 1.3×10^6 km^2 and caused damage over an area of about 80,000 km^2 within which the most frequently occurring intensity did not exceed VII (MM). It mostly affected the densely populated and rapidly developing centres of Craiova, Pitesti, Zimnicea, Bucharest, Ploesti, Focsani, Birlan and Iasi, where it caused damage worth over 10^9 $ US. The earthquake killed about 2,100 people of whom 1,800 perished in Bucharest (BU), and injured 10,500, 2,300 seriously. Much of the damage was caused to old multi-storey reinforced concrete buildings of 6–12 storeys. Some of them had been damaged in 1940 by an earthquake of almost identical characteristics and by the war. Relatively few modern high-rise constructions suffered serious structural damage.

In Bucharest, at a focal distance of 190 km, the shock triggered an SMAC-B strong-motion accelerograph and a Wilmot seismoscope in the basement of a single-storey structure founded on thick, compact alluvium. The SMAC record shows that the strongest shaking began about 19 s after the instrument started to record, and this strongest shaking consisted of a double acceleration pulse in the two horizontal components of 154 and 206 gal respectively that contain more than 80% of the total energy flux of the event. Figure 1 shows the acceleration response spectrum (S_a), for 5% critical damping, of the main portion of the north–south component of motion[1]. It also shows the response spectra of two other ground motions produced by earthquakes of roughly the same magnitude, in the near-field (El Centro, EC) and in the far-field (Mexico City, MX) for comparison. The former occurred on 19 May 1940 at a depth of about 7 km, had a magnitude $M = 7.1$ and was recorded on dense alluvium at a focal distance of 14 km. The latter occurred on the 11 May 1962 at a depth of about 40 km, had a magnitude $M = 7.0$ and was recorded on very soft and thick deposits of high water content at Mexico City, at a focal distance of 270 km. The difference in shape of the response spectra between far-field (BU and MX) and near-field (EC) as well as the shift of the spectral maxima is obvious. This difference is more apparent in Fig. 2 where the response spectra of Fig. 1 have been normalised with respect to their corresponding zero period accelerations. Allowing for the larger magnification in the MX record for periods longer than 2 s, due to layer

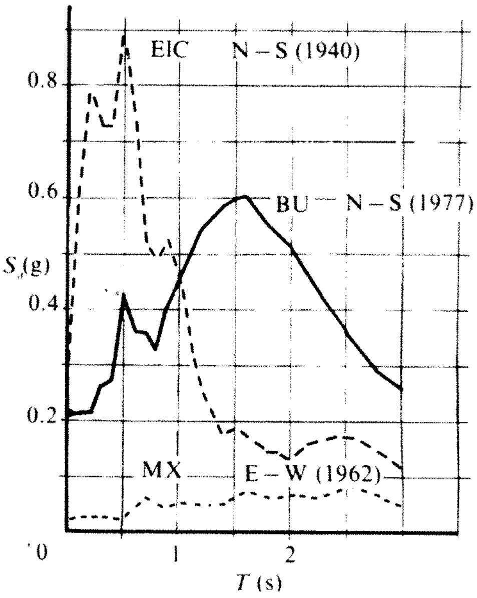

Fig. 1 Acceleration response spectrum of the main portion of the north–south component of motion in the Bucharest earthquake. The ground motions produced by other earthquakes in El Centro and Mexico City are shown for comparison.

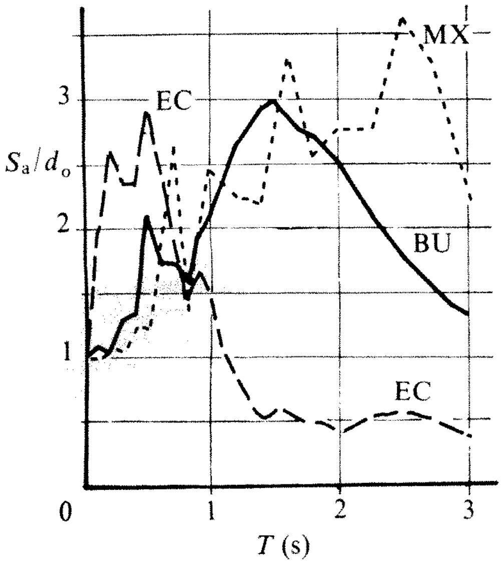

Fig. 2 Normalised spectra from Fig. 1 with respect to their corresponding zero period accelerations.

response, all three events show the same maximum amplification of about three times their zero period acceleration but at distinctly different periods, much longer for the far-field motions.

There is, of course, insufficient evidence to claim that the (BU) spectra are representative of an intermediate depth, medium magnitude earthquake. They simply imply that in this particular case the focal mechanism and preferential attenuation at higher frequencies along the path produced intense ground motions at unusually high periods.

These long-period characteristics of intermediate-depth earthquakes from the Vrancea region are reflected also on the seismoscope recordings. In Bucharest, the recording of the Wilmot seismoscope shows a double amplitude of 41 mm which is consistent with 44-mm amplitude response of the SMAC-B record at $T = 0.75$ s and 10% damping. At Galati, at a focal distance of 140 km the double amplitude was 27 mm; at Nis and Skopje in Jugoslavia, at distances of 480 and 610 km, the Wilmot amplitudes were about 6 and < 1 mm respectively. Other earthquakes originating from Vrancea, those of 2, 15 October and 14 December 1966 of magnitude 5.6, 5.4 and 5.2 produced seismoscope amplitudes at Ksihinev in the USSR, at a focal distance of 260 km, of 1.7, 1.4 and 0.7 mm respectively.

The greater effect of the 1977 earthquake in Bucharest was on flexible, 6–12-storey high reinforced concrete frame structures of low ductility without shear walls. These structures have a fundamental period of the order of 0.7–1.6 s, which places them on the ascending branch of the BU spectrum. Progressive damage during the earthquake should have caused a lengthening of their period and an increase in the lateral forces acting on them, whereas for the EC spectrum just the opposite is true. In contrast, rigid structures of large panel or frame construction with shear walls, of the same height, as well as 1–3-storey masonry dwellings, suffered little damage. These structures have much shorter periods of the order of 0.2–0.7 s, and they belong to the early, more subdued part of the BU spectrum. Had the ground motions in Bucharest been of the EC type, the greater effects of the earthquake should have been on the more rigid structures.

It is evident, therefore, that the adequacy of current lateral force code requirements derived from near-field ground motions, may be insufficient for flexible, low ductility structures wherever there exists the potential of a large, distant or intermediate-depth earthquake. This situation may arise more often than previously believed, particularly in the eastern Mediterranean region, and in fast developing cities at some distance from major throughgoing faults capable of producing large earthquakes.

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Confirmation of central volcanoes off the Icelandic coast

The Icelandic shelf and slope region occupies much of the transition zone between the complex Icelandic hot spot and more uniform ocean-ridge areas to the north and south. This transition zone is relatively little known geologically, but in this communication we summarise some recent observations on the nature of its basement. It is well known[1] that the shelf is generally narrower off the eastern half of Iceland than off its western half. This is most obvious south of Iceland, where the shelf width changes suddenly at the eastern volcanic zone; the insular slope is also steepest off south-eastern and north-eastern Iceland. Recent geophysical surveys[1,2] in the southern and south-eastern shelf area have indicated the presence of an abrupt basement scarp now covered by sediments, 5–14 km inside the bathymetric shelf break (Fig. 1). This scarp is at least 1 km high and only a few km in mean width. No such basement scarp is indicated in comparable surveys conducted so far off western Iceland[1–3]; north-east of the island, detailed data is not yet available.

A causal relationship between this and other observed east–west asymmetries in the geology of Iceland, including the much greater uplift and erosion in eastern than in western Iceland since the Upper Tertiary, is likely to be found in differences in crustal thickness or upper mantle composition respectively east and west of the eastern volcanic zone. Similarly, the abrupt change in the chemistry of recent volcanics occurring north of Iceland[4] very close to the shelf break, may directly reflect changes in crustal thickness[5], rather than damming effects of the Tjörnes fracture zone on subcrustal flow[4].

Another point concerns the central volcanoes of Iceland, first described by Walker[6], which are of major significance in its geological evolution. Over 60 such centres, active or extinct, have now been identified on the island. From the results of geophysical surveys[3,7] it has been concluded that several central volcanoes occur on the insular shelf off eastern and western Iceland, and that the trace of the Icelandic hot spot in the North Atlantic may coincide with the greatest density of such centres.

In an attempt to confirm the occurrence of central volcanoes on the Icelandic shelf, we have dredged at three sites (Fig. 1) where topographic highs are associated with the major magnetic anomalies believed to be characteristic for such volcanoes. The dredging was carried out by the RV Hafthor, of the Marine Research Institute, in November 1975, and a collection of igneous rock boulders was recovered at each dredge site.

Those features of a dredge collection which might be used to indicate the presence of a central volcano include (1) a large proportion of acidic, intermediate or gabbroic rocks (2) rocks having suffered intense hydrothermal alteration, and (3) unusually magnetic and magnetite-rich rocks. Accordingly, the rock types in these dredge samples were identified in thin section, and their magnetic susceptibility and natural remanence were measured in core specimens. The results (to be published in more detail[8]) may be summarised as follows.

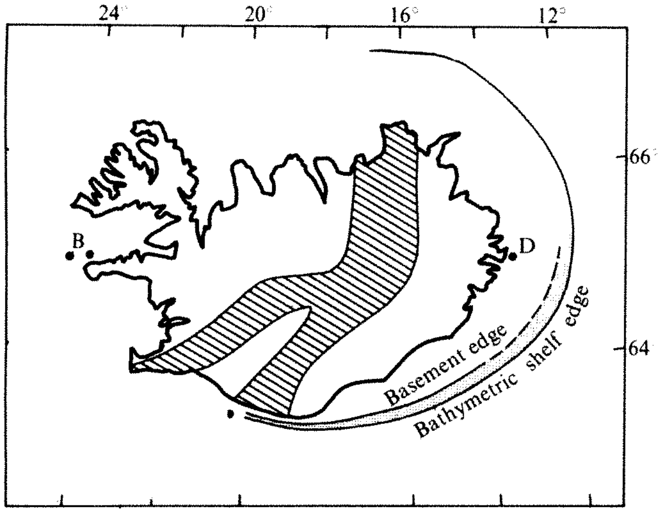

Fig. 1 Map of Iceland, showing the smoothed position of the upper edge of the steep insular slope off the eastern half of the island, and a major basement edge inferred from marine magnetic and gravity data off southern and south-eastern Iceland[1,2,7] to occur under shelf sediments. Both edges are indistinct between 64° and 65°N. Hatched areas: active volcanic zones in Iceland. B, D, Dredge localities.

Of the two dredge sites off western Iceland (20 km apart), one yielded six basalt boulders and six rhyolite boulders, while the other yielded 71 basalt and diabase boulders and two rhyolites. Alteration state and magnetic properties of these samples are similar to those found in lavas on shore.

At the Diönubodi dredge site 14 km off Gerpir promontory, eastern Iceland, the samples recovered were predominantly (21 out of 35) of acid and intermediate rock types, including granites, rhyolites, acid tuff, and andesites. Two samples contain epidote. The magnetic properties of this collection are also unusual: thus, nine specimens out of the 34 measured have volume susceptibilities exceeding 0.1 T A^{-1} m (8×10^{-3} G Oe^{-1}), compared with only 1% of the specimens in a collection from 740 Eastern Iceland basalt lavas recently measured by us.

We are confident that the Diönubodi dredge collection can only be derived from a local central volcanic complex, which also causes the large (up to 6,000 nT) magnetic anomaly[7] observed there. It is, furthermore, difficult to explain the high proportion of acid rocks at one of the western sites without recourse to similar conditions. These findings strongly support previous hypotheses[3,7] on the occurrence of other central volcanoes on the Icelandic shelf.

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How do children learn the meanings of verbs?

ONCE children have begun to learn their native tongue, they acquire vocabulary at a remarkable rate. An intelligent 6-yr-old is able to recognise 13,000 words, and more than double that number 2 yr later[1]. According to orthodox linguistic theory, the learning of verbs and other relational terms ought to be difficult because, in addition to their meaning, they have 'selectional restrictions', which constrain the noun phrases that can occur with them[2]. A child must learn, for example, both the meaning of the verb 'fly' and the fact that it takes subjects which are restricted to denoting moveable objects. This linguistic theory has, however, been criticised, and it may be a misleading basis for a model of language acquisition since meaning and selectional restrictions can hardly be independent[3]. In particular, the subjects and objects that occur with a verb might help the child to infer its meaning, and likewise the meaning of a verb might help to define its possible subjects and objects.

In order to investigate such assumptions, we provided 15 young children (mean age, 3.10 yr) with an opportunity to learn some verbs. On each trial, the experimenter told a story to one of the children and acted it out with puppets. A typical story ran as follows: 'John stepped out of the boat and the water mibbed his trousers so he went home to change into some dry clothes. The water had mibbed his trousers right through so Simon made him some hot tea. But John dropped his cup and the tea mibbed over the floor'. The novel verb, mib, has a transitive meaning similar to that of soak and an intransitive meaning similar to that of spill—there is no single word corresponding to it in English, but it always occurred with a subject noun phrase denoting a liquid. The child heard the story four times: on the first two occasions the experimenter acted it out appropriately, and on the second two occasions the child attempted to do so. As a test of what the child had learned, he or she was presented with four items unrelated to the story (for example a female doll, some orange juice in a container, a car, a ball) and asked 'Which one can mib?' Only one item—the orange juice—is in accord with the selectional restrictions of the verb. Any child who made a correct choice was retested 7–10 d later. In this retrial, only the test was carried out: the child was simply asked to pick out the item that mibbed from a new set of four.

Four different stories were devised in order to test the children's ability to acquire four sorts of verb defined in terms of their selectional restrictions: animate subject, animate object, liquid subject, liquid object. Earlier studies of children's comprehension of sentences suggested that animate subject and liquid object verbs should be easier to learn than liquid subject and animate object verbs[4,5]. A fifth story was also used for reasons that will become apparent shortly. None of the novel verbs had any single semantic counterpart in English. They were based on five nonsense syllables, with association values of approximately 2.1 (ref 6), which were allocated to the stories in a counterbalanced way from one subject to another. Each child was individually tested with each of the stories in five separate sessions during the course of a morning. The order of presentation of the stories was randomised.

The children picked out a mean of 2.4 correct items on the five initial tests (with eight children performing significantly better than chance). There were 33% correct responses for animate subject verbs, 66% for animate object verbs, 60% for liquid subject verbs, and 46% for liquid object verbs. These differences fail to support the view that animate subject and inanimate object verbs should be easier to learn, and were not significant (Cochran's $Q = 2.42$, $P > 0.50$). A week later, the children who had responded correctly to a verb were retested with it, and the percentages of correct responses were: 76% for animate subject verbs, 66% for animate object verbs, 12% for liquid subject verbs, 28.5% for liquid object verbs. The animate verbs were significantly better retained than the liquid verbs (Fisher–Yates exact probability, $P = 0.01$, on the assumption that all the data were independent).

Selectional restrictions probably involve an inference about whether a particular referent is a possible subject of a verb, and so on[7]. Hence, in the case of a sentence such as, 'The Smiths saw the Rocky Mountains flying to California', a child who lacks some pertinent general knowledge about mountains or relative motion will be unable to make the required inference and may well conclude that the Rocky Mountains were flying. In order to test this hypothesis, a fifth story was constructed with a nonsense verb that selects subjects capable of movement. As a test of what had been acquired, the children were asked to act out two sentences: 'Jane saw the boat gebbing towards the ball', and 'John saw the house gebbing towards the park'. Their initial performance was 93% and 33% correct, respectively. When we retested the successful children a week later, performance was 100% and 50% correct, respectively. Evidently, it was easy for the children to learn that the subject of the nonsense verb denoted something that moved, but few children made the intended interpretation of the second test sentence. Its syntax suggests that the house is the entity that gebs and, in fact, 10 subjects attempted to move the house. They did not infer that the house cannot move with respect to the park. One subject, however, put the doll in front of the house with his hands in the air and explained: "He's stopping the house from gebbing to the park".

At least half the children learned something about the selectional restrictions on verbs from the stories they heard, which suggests that this experimental procedure is useful. But it does provide two clues to the meanings of the verbs: the accompanying actions and the linguistic context. A second study was accordingly carried out in which 14 children (mean age 4.3 yr) merely listened to the stories (4 times), which were not accompanied by any actions. Performance on the initial tests corroborated the results of the previous study: 11 subjects made two or more correct responses which, overall, were very evenly distributed across the four sorts of verb. There was also no significant difference, however, between the verbs on the retest a week later.

Young children are evidently capable of picking up incidental information about a variety of different sorts of verb. In previous studies of comprehension, selectional information has often been uncontrolled. For example, it has been found that 5-yr-olds tend to answer the question, 'Is this doll easy to see?', as though the doll were the subject of the verb. It remains unclear whether the response reflects a genuine syntactic bias[8] or merely the fact that the major selectional restriction of the verb clearly points to an entity with eyes.

Our results suggest that children are sensitive to whatever cues are available to meaning. They readily pick up selectional information about both animacy and liquidity. With hindsight, it might be odd if this tacit skill were exercised to a significantly different extent with such familar concepts. Their ability to retain newly acquired knowledge may, however, depend on its nature and on the sort of experience leading to its acquisition. The answer to our question does, indeed, seem to be that children learn the meanings of verbs rapidly, without their attention being deliberately drawn to them, and often in circumstances where the major cue is the selectional information provided by the sentence as a whole.

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Adoption study supporting genetic transmission in manic–depressive illness

Comparison of adoptive parents of persons with a psychiatric disorder with their biological parents provides a unique opportunity to separate the interacting aetiological roles of heredity and environment. We have conducted such a study in manic–depressive illness; the results strongly support the importance of genetic factors in the transmission of this disorder.

A genetic vulnerability to manic–depressive disorder has been demonstrated by family, twin, and linkage studies[1–4]. To our knowledge, this is the first report of an adoption study of affective illness, but research involving adoptive families has been done for schizophrenia and other psychiatric disorders[5–10]. Our investigation was carried out in Belgium because adoptive registers are available there, and the adoption agencies were willing to help us to obtain subjects.

Our subjects were the adoptive and biological parents of manic–depressive adoptees. There were three sets of controls: (1) the parents of manic–depressives who were not adoptees; (2) the adoptive and biological parents of normal adoptees, and (3) the parents of individuals who had contracted poliomyelitis during childhood or adolescence. This last group was designed to control for the effect on parents of bringing up a disabled child.

The group of affected offspring (that is, manic–depressive adoptees) was ascertained by systematically examining the medical records of five outpatient clinics and five inpatient services in the vicinity of Brussels. All admissions during a 5-yr period (1971–1976) were reviewed. Those persons who were designated as manic–depressive and who were described as having been adopted were interviewed by one of us (J.M.) to

Table 1 Demographic characteristics of the offspring

	Index cases	Controls		
	Manic–depressive adoptees $N = 29$	Manic–depressive non-adoptees $N = 31$	Normal adoptees $N = 22$	Polio $N = 20$
Ascertainment				
Adoption Agency	—	—	12	—
Inpatient	19	17	—	—
Outpatient	10	14	—	20
Age (mean, yr)	32.41	32.35	32.00	29.25
Sex				
Male	10	9	6	5
Female	19	22	16	15
Age at adoption (mean, in months)	5.41	—	6.00	—
Age of onset (mean)	22.07	24.26	—	8.35
National origin				
Flemish	16	18	13	13
Walloon (French)	13	13	9	7
Religion				
Catholic	25	26	22	17
Protestant	4	5	0	3
Socio-economic status*				
Education (mean)	3.83	3.90	3.86	3.80
Occupation (mean)	3.90	4.00	3.95	3.80
Marital Status				
Married	16	21	16	8
Single	10	5	4	12
Divorced	3	5	1	0
Separated	0	0	1	0

* Hollingshead–Redlich scale[13].

Table 2 Demographic characteristics of the parents

	Adoptive of manic–depressive adoptees (*N* = 29)			Biological of manic–depressive adoptees (*N* = 29)			Biological of manic–depressive non-adoptees (*N* = 31)			Adoptive of normal adoptees (*N* = 22)			Biological of normal adoptees (*N* = 22)			Biological of polio patients (*N* = 20)		
	♂	♀		♂	♀		♂	♀		♂	♀		♂	♀		♂	♀	
Number living	29	28	(57)*	29	28	(57)	31	30	(61)	21	21	(42)	21	22	(43)	19	20	(39)
Age (mean, yr)	61.9	61.2		56.8	55.7		59 9	57.1		59.1	58.1		54.9	52.5		52.8	50.3	
Socio-economic status†																		
Education			3.86			(4.93)			(4.00)			3.95			(4.54)			(3.70)
Occupation			3.93			(4.93)			(4.26)			4.00			(4.59)			(3.75)
Marital Status																		
Married to each other			29			(9)			(27)			22			(2)			(17)
Married to different spouses			0	(15)	13)				(0)			0	14	16				(0)
Never married			0	2	4				(0)			0	5	3				(0)
Divorced			0			(2)			(3)			0			(1)			(2)
Separated			0			(1)			(1)			0			(0)			(1)

* Figures in parentheses are totals.
† Hollingshead–Redlich Scale[13].

confirm the diagnosis. We used a modified form of the semi-structured interview, Current and Past Psychopathology Scales (CAPPS) (ref. 11) and the diagnostic criteria of Feighner *et al.*[12]. Only persons who had bipolar illness (that is, had experienced both manic and depressive episodes) were accepted as probands. To meet our criteria, they also had to have been transferred to their adoptive homes before 1 yr of age and to have been raised by those parents until adulthood. The adoption agencies supplied the details on the dates of adoption of the offspring, and gave us the names of the biological parents. Municipal registers were used to locate all parents. We only accepted families in which at least three of the four parents were available and willing to participate.

Manic–depressive offspring who were not adopted were ascertained from admissions records of the same facilities as the adoptees, covering a similar time period. Psychiatrically normal adoptees were ascertained through the adoption agencies; these individuals were given clinical interviews and only those without psychopathology were accepted. Their biological and adoptive parents were traced by the same procedure as those of the experimental group. None of the adoptive parents were related to the biological parents. The poliomyelitis patients were ascertained through three paediatricians who had been treating them over a long period. The three groups of control offspring were selected out of the total number of possible subjects by matching them with the index population according to age, sex, national origin, religion, and socio-economic status. Table 1 summarises the ascertainment and demographic characteristics of the experimental and control groups.

All of the parents were interviewed by one of the clinicians, blind as to the adoptive and clinical status of the offspring. The same instruments and criteria were used as for the diagnostic confirmation of their offspring. The interviews took place in the parents' homes unless the parents preferred to meet at the local centre. The greatest distance travelled was 52 miles from Brussels, indicating a low mobility of this sample. Two and a half to three hours were spent with the parents for the psychiatric interview. The parents were instructed at the outset not to speak about their children until specifically asked. Demographic characteristics of the parents are summarised in Table 2.

The major finding of this investigation is that psychopathology in the biological parents is in excess of that found in the adoptive parents of the same manic–depressive offspring (Table 3). If we focus on disorders which we may call the affective spectrum, namely, bipolar affective disease (episodes of mania and depression), unipolar affective disease (psychotic depressions without mania), schizoaffective psychosis (schizophrenic and affective episodes), and cyclothymia (cyclothymic personality), the difference is significant at the level of $P < 0.025$ ($\chi^2 = 5.10$). Previous genetic studies by us and others support the inclusion of unipolar and schizoaffective disorders as genetically related to bipolar illness when they are found in close relatives of bipolar patients[2,4,14–16]. If we include other forms of psychopathology, the difference is highly significant ($\chi^2 = 7.29$, $P < 0.01$). The frequency of non-affective psychopathology was no higher in the biological parents of bipolar adoptees (9%) than in those of normal adoptees (16%), indicating that it is by virtue of the affective disorders that the former differ from the latter (31% compared with 2%).

Table 3 also shows that the degree of psychopathology in the biological parents of manic–depressive adoptees is similar to that of the parents of the non-adopted manic–depressives, and that the rate of psychiatric disorder in the adoptive parents of the experimental group is similar to that of the adoptive parents of the normal offspring group. The degree of total psychopathology in the biological parents (of normal offspring) who gave their children for adoption is slightly greater than in adoptive parents who brought up those same individuals. It is

Table 3 Diagnosis of the parents

	Bipolar adoptees (*N* = 29)						Bipolar nonadoptees (*N* = 31)			Normal adoptees (*N* = 22)						Poliomyelitis (*N* = 20)		
	Adoptive parents			Biological parents			(Biological parents)			Adoptive parents			Biological parents			Biological parents		
	♂	♀		♂	♀		♂	♀		♂	♀		♂	♀		♂	♀	
Bipolar	1	0	(1)*	3	1	(4)	2	0	(2)	0	0	(0)	0	0	(0)	0	0	(0)
Unipolar	3	3	(6)	1	11	(12)	3	8	(11)	1	2	(3)	1	0	(1)	3	1	(4)
Schizoaffective	0	0	(0)	0	2	(2)	1	0	(1)	0	0	(0)	0	0	(0)	0	0	(0)
Cyclothymic	0	0	(0)	0	0	(0)	1	1	(2)	1	0	(1)	0	0	(0)	0	0	(0)
Affective spectrum	4	3	(7)†	4	14	(18)†	7	9	(16)	2	2	(4)	1	0	(1)	3	1	(4)
Percentage	14	10	(12)	14	48	(31)	23	29	(26)	10	10	(10)	5	0	(2)	15	5	(10)
Schizophrenia	0	0	(0)	0	0	(0)	0	1	(1)	0	1	(1)	0	0	(0)	0	0	(0)
Alcoholism	2	0	(2)	2	1	(3)	1	1	(2)	0	0	(0)	1	2	(3)	1	0	(1)
Sociopathy	0	0	(0)	1	1	(2)	0	1	(1)	0	0	(0)	2	1	(3)	0	0	(0)
Other	0	0	(0)	0	0	(0)	0	0	(0)	0	0	(0)	0	1	(1)	0	0	(0)
All psychopathology	6	3	(9)‡	7	16	(23)‡	8	12	(20)	2	3	(5)	4	4	(8)	4	1	(5)
Percentage	21	10	(16)	24	55	(40)	26	39	32	9	14	(11)	18	18	(18)	20	5	(12)

* Figures in parentheses are totals
† $x^2 = 5.10, P < 0.025.$
‡ $x^2 = 7.29, P < 0.01.$

worth noting that this difference is due to an excess of alcoholism and sociopathy in the former group. Finally, the degree of psychopathology in the parents of polio patients is in the same range as in both groups of adoptive parents. All these findings support our conclusion that our experimental sets of parents are truly representative of the degree of psychiatric disorder which is present respectively in those parents who bring up and those who contribute genetically to manic–depressive individuals.

Another finding of interest is that no father-to-son transmission of manic–depressive illness was seen in our entire sample. This is consistent with a sex-linked model of bipolar illness, which has been suggested by some previous genetic studies[2–4]. Finally, bipolar illness, as would be expected, had an early onset in all parents in whom it was present; however, the onset of unipolar illness in the adopting parents occurred, in every case, after the onset of manic–depressive illness in their children, whereas in the biological parents, onset of unipolar illness occurred almost always previous to the onset in their children. This observation strengthens the genetic hypothesis by suggesting that the adoptive parents' unipolar illness might be more reactive and less severe than that of their biological counterparts; early onset is often considered to be an index of severity in psychiatric disorder.

In summary, we found a greater degree of psychopathology, particularly of affective illness, in parents genetically related to manic–depressive probands than in parents who adopted and raised these same individuals. The results demonstrate the importance of genetic factors in the aetiology of manic–depressive illness.

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Number of trophic levels in ecological communities

ECOLOGICAL food chains are typically short, consisting of not more than four or five trophic levels. This is usually explained by a reduction in the energy which is available to successive links in the food chain[1,2]. In contrast, we believe that the number of trophic levels is constrained by population dynamics and not by ecological energetics.

In the extreme, food chains must obviously be limited by the flow of energy. Slobodkin's[2] hypothetical predator occupying a trophic level higher than twenty and needing a continent to support it is a dramatic example. However, if food chains are primarily limited by energy they should be longer in more productive ecosystems; for example with an ecological efficiency[2] of approximately 10%, trophic levels should increase at the rate of one for each tenfold increase in the energy entering the system. This does not seem to be true. Primary productivity ranges over at least four orders of magnitude in terrestrial ecosystems, over three for aquatic systems[3]; yields of fish and the productivity of animal populations range over five or more orders of magnitude[4,5]; yet the number of trophic levels supported by these particular plant and animal populations does not seem to be closely correlated with the productivity of the base of the food chain. Food chains are not noticeably shorter in barren Arctic and Antarctic terrestrial ecosystems[6,7] compared with a productive tropical savannah[8] or the fish guilds of a tropical coral reef[9].

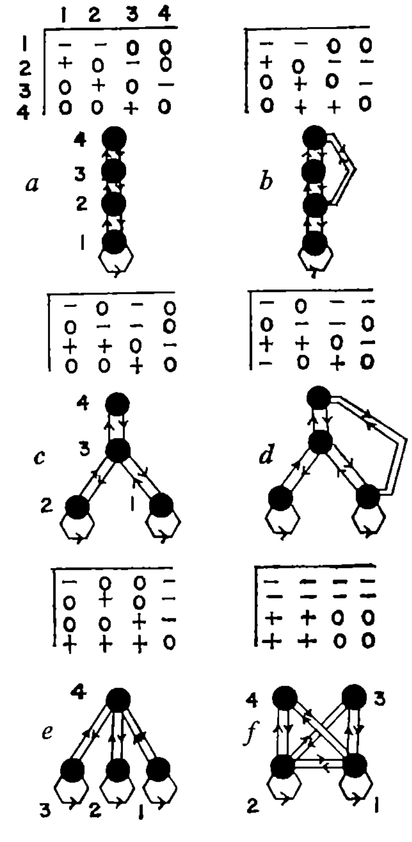
One explanation for short food chains is that it is advantageous to feed low in the chain, where more food is available. The effects of body size[1], or the seasonality and predictability of resources may also be important[10,11]. However, the dynamically fragile nature of long food chains does not seem to have been discussed.

Consider a system of n interacting species described by equations of the form

$$\frac{dX_i}{dt} = X_i\left(b_i + \sum_{j=1}^{n} a_{ij}X_j\right) \equiv F_i \qquad (1)$$

where X_i is the population size and b_i the instantaneous rate of growth of the ith species, and a_{ij} is the *per capita* effect of the

Fig. 1 The matrices show whether the species along each row has a positive (+), negative (−) or no (0) effect on the species in each column at equilibrium; the figures below indicate these relationships diagrammatically.



jth species on the ith species. We used equation (1) to construct model food chains of varying length and complexity, and judged their feasibility by whether or not they were locally stable, and if they were, how long they took to return to equilibrium after a disturbance (the 'return time'). Long return times imply an increased probability of extinction in the real world[12–14]. The dynamics of equation (1) near equilibrium are determined by the eigenvalues of a matrix which is the Jacobian

$$\frac{\partial F_i}{\partial X_j} \begin{matrix} j = 1, n \\ i = 1, n \end{matrix}$$

evaluated at equilibrium. A system which contains non-negative eigenvalues will be locally unstable, while the return time of a locally stable system will be roughly proportional to the reciprocal of the largest eigenvalue. Formally

$$\text{Return time} = -1/\text{Real}\,(\lambda_{\max}) \text{ (for all } \lambda < 0)$$

In general, as the number of species in a model ecosystem increases, the probability of local stability decreases[15]. But it is possible using differential equations to construct linear food chains of any length that are locally stable (Fig. 1*a*), and Maynard Smith[16] has argued that "the lengthening of food chains will not by itself cause instability". This view gives a distorted impression of what is biologically feasible, because as the number of trophic levels increases, so do return times.

We have followed established practice[12,17–19], and investigated eigenvalues of matrices whose elements are random variables subject to constraints of sign and absolute magnitude. The possibilities investigated are shown in Fig. 1. Parameter values were set by the biological details implied by the model[13]. The diagonal elements of the Jacobian are $X_i^*a_{ii} = -b_iX_i^*/K_i$ where X_i^* is the equilibrium population of the ith species, and K_i its equilibrium in the absence of all other species. Since the X_i^* cannot exceed K_i these values will be in the interval $(0, -b_{ii})$ (the b_{ii} were set equal to 1 in each species). The off diagonal elements are also of the form $X_i^*a_{ij}$ where the sign of a_{ij} will depend on whether i is feeding on j or vice versa, or whether i and j are competitors. For predator–prey interactions (Fig. 1 *a–e*) a_{ij} is the number of new predators produced per prey eaten; the latter will usually be much larger than the former. The X^* will also differ, since predators are likely to be fewer than their prey at equilibrium. The effects of the predator on the prey population were therefore uniformly distributed on the interval $(0, -10)$ and the effects of the prey on the predator on the interval $(0, +0.1)$. For competition (Fig. 1*f*) $X_i^*\cdot a_{ij}$ is the magnitude of the interspecific competition. This will usually be less than the absolute value of the intraspecific competition $(X_i^*\cdot a_{ii})$ and was uniformly distributed on the interval $(-X_i^*\cdot a_{ii}, 0)$. For each of the six configurations of signs shown in Fig. 1 nearly 2,000 matrices were created whose elements satisfied these general constraints. Figure 2 shows their return times. (Results were qualitatively the same in 1,000 initial simulations in which the matrix elements had absolute values between zero and one, suggesting that they are not sensitive to the assumptions about the magnitude of the matrix elements.)

Consider first the models without omnivores (Fig. 2*a*, *c*, *e*). As the number of trophic levels increases, so do return times. For two trophic levels, nearly 50% of the models had return times less than or equal to five; with three trophic levels, this drops to 5% and with four, to less than 1%. Figure 2 also shows the percentage of models where return times exceed 150. With two trophic levels this is very small (0.1%), but is 9% and 34% respectively with three and four trophic levels.

A two-species system with one predator and one prey can be examined analytically. Here there are as many species as trophic levels, analogous to model (*a*) of Figs 1 and 2. About 70% of the two-species models had return times less than or equal to five—a considerably larger percentage than is found in model (*a*), but comparable with the situation in model (*e*). Hence, models with different numbers of species and the same number of levels, and models with the same number of species but different numbers of levels lead to similar conclusions. We suggest that this increase in return times with an increasing number of trophic levels will play an important part in limiting the lengths of food chains subject to the perturbations of the real world.

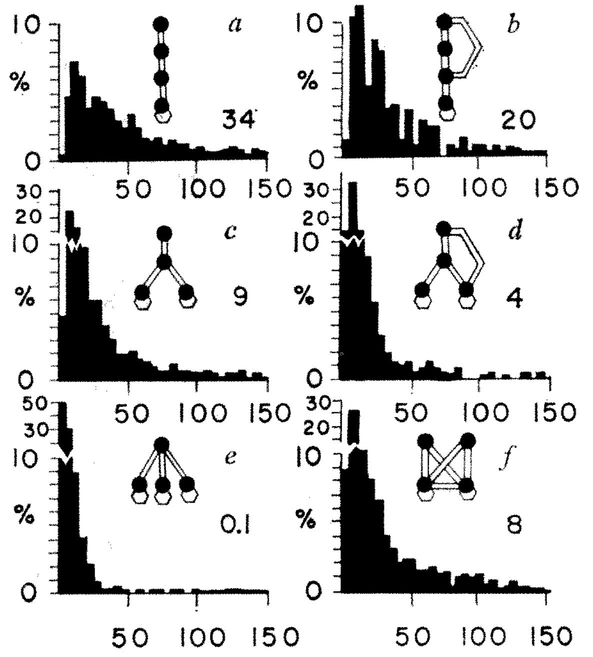


Fig. 2 The distribution of the reciprocals of the largest eigenvalues (a measure of return times) from nearly 2,000 matrices of each configuration. The number on each histogram is the percentage of those reciprocals which have a value greater than 150.

With omnivores, 78% of both the three (Fig. 1*d*) and four (Fig. 1*b*) trophic-level models are unstable. Return times in the remaining 22% are illustrated in Fig. 2*b* and *d*, which show that omnivores do not affect the conclusion that longer food chains imply longer return times; however, the distributions are shifted slightly towards faster values. Whether or not the addition of omnivores (further complexity) contributes to the 'stability' of a system then depends on how these results are evaluated. For systems assembled at random, omnivory increases the probability of instability; however those that are locally stable have faster return times, and in this sense are more stable. This paradox does not seem to have been mentioned in studies on 'complexity' and 'stability' in model ecosystems.

Figure 2*f* includes competitive effects in the lowest trophic level. As expected[20], 53% of the models were unstable but the return times of the remainder were no greater than in model (*c*), a three-trophic-level model, which lacks the destabilising factor of competition. However, it is known that a 'pure' competition community (one trophic level) of n interacting species may have more than one locally stable equilibrium[21]. It is not clear how the addition of higher trophic levels would influence the existence of, and rates of return to alternative equilibria; further work is required on this point.

We have only considered one type of model (differential equations) and one aspect of their dynamic behaviour (return time). It is easy to construct very different models, implying different biological details, and with very different behaviours. It is therefore encouraging to note that in a discrete generation (difference equation) model of an insect host–parasitoid interaction (two trophic levels), large areas of stable parameter space disappear when a third trophic level (a hyperparasite) is added[22]. This reinforces our view that the number of trophic levels in ecological communities is determined not by ecological energetics, but by population dynamics.

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Large benthic microbial communities in sulphide biota under Peru–Chile Subsurface Countercurrent

BENTHIC observations off the coast of Chile have consistently disclosed the presence of large coherent microbial communities living at depths of about 50–280 m in the H_2S-containing sediments of the shelf in contact with the deoxygenated waters of the Peru–Chile Subsurface Countercurrent (SCC). Similar observations were also made off Peru in 1969 by Gilbert T. Rowe, and in 1976 by G. T. Rowe and John Waterbury of Woods Hole Oceanographic Institution. The microflora, which has only been reported once before in the literature[1], has been known for years by the local fishermen who call them *estopa* (Spanish for uncleansed wool or fiax) due to the filamentous appearance of its main components. In this report I describe this massive microbial community which includes organisms typical for sulphide biota, and may have unsuspected importance in the ecology and economy of the sea off western South America.

Repetitive quantitative grab sampling off Concepcion, Chile (36°35′30″S, 73°04′20″W) at 60-m depth, indicated biomass of 106 g (wet weight) per 0.1 m^2 for the microbial component, while the benthic infaunal biomass in the same sample attained only 11.5 g (wet weight) per 0.1 m^2 (Peterson 0.1-m^2 sample washed through a 0.25-mm^2 sieve). Preliminary examination of the community has shown that it consists of many kinds of prokaryotes. The main constituent, however, is typically large filaments of *Thioploca* spp. This genus of gliding bacteria has not been previously recorded in open oceans. The *Thioploca* spp. fall clearly into three groups according to their cell diameter: (1) 30–40 μm, (2) 15–20 μm, and (3) 2.5–5 μm, (Dr Maier, Ohio University, personal communication). Other members of the community are *Oscillatoria*-type blue-green algae (cyanobacteria), flexibacteria (probably of the genus *Chlorophlexis*) and various other forms of bacteria.

The *Thioploca*-like forms appear as many individual filaments within a common sheath (Fig. 1, inset) forming large, whitish-yellow, twine-like structures that attain 100–500 μm in diameter and several cm in length, therefore perfectly visible to the naked eye (Fig. 1). Off the Bay of Concepcion, where most of the recent observations have been carried out, the sediments consist of a loose superficial yellowish-brown layer of a few mm thick, an intermediate layer of black H_2S-containing sediment about 8 cm thick, and a lower dark-brown layer of sticky compacted mud[1]. The filamentous microbial material occurs in the upper two layers, giving the sediment a soft, spongy texture. The sediment, a diatomaceous mud, also contains large quantities of assorted organic debris such as fish scales, fish bones, faecal pellets, few shell fragments, empty polychaete tubes and some terrestrial vegetal remains.

Although the genius *Thioploca* is classified among the colourless Beggiatoaceae[2], some species of the genus contain a greenish blue pigment and have therefore an uncertain taxonomic relationship to the *Beggiatoa*[3]. Ethanol extracts from seawater-washed filaments have given absorption spectra typical for chlorophyll *a*, with major peaks at about 410 nm and 670 nm; however, it is probable that this pigment is associated with oscillotorian cyanobacteria known to be components of this community. The gliding of independent filaments occurs within their common sheath at speeds of up to 12.5–25 μm min^{-1}.

Whatever the taxonomic position of these *Thioploca*-like forms, their large standing-crop suggests an important role in the ecology of the upwelling biome off southwestern South America.

The SCC, a wedge-shaped water mass distributed between northern Peru and southern Chile (to about 41°S), is characterised by relatively high salinity and temperature, and extremely low oxygen content, typically under 0.1 ml O_2 per l. Nutrient concentrations are high[4–6].

The presence of filamentous microbial associations should be looked for in other parts of the world where similar oceanographic conditions exist. Recently a single *Thioploca*-like filament was observed in a small sediment sample collected off Walvis Bay in

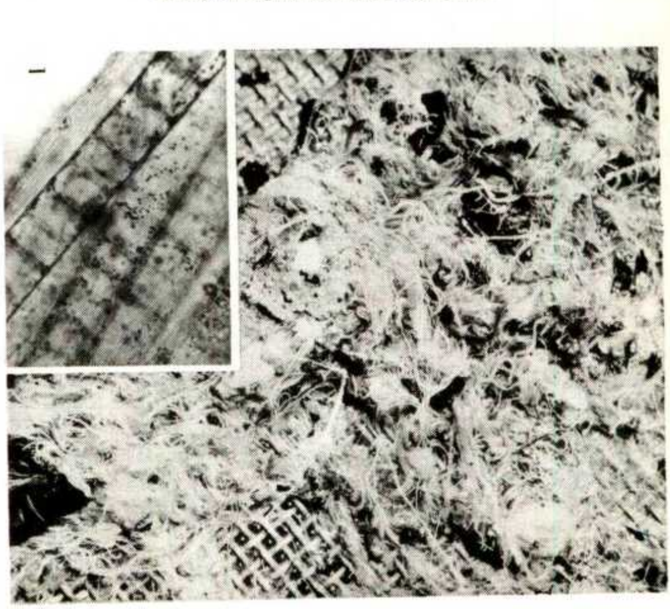

Fig. 1 Filamentous microflora deposited on a 1-mm^2 mesh 12-inch diameter geological sieve after washing a 0.1 m^2 Petersen grab sediment sample collected at 100-m depth off the Bay of Concepcion, central Chile, October 7 1975. At 90 m salinity was 34.54‰, temperature 9.71 °C, and dissolved oxygen 0.54 ml l^{-1}. Inset, photomicrograph of portion of *Thioploca*-like form no. 1. The common sheath and four cell-chains containing intracellular sulphur granules are visible. Cell dimensions are 2436 μm diameter and 3036 μm length. Bar represents 10 μm.

south-western Africa (J. W. Farrington). In this upwelling region a mysterious benthic 'slimy grass' has been reported at depths of about 90–130 m (ref. 7), occurring with a low-oxygenated subsurface countercurrent impinging against the shelf[8].

The coincidence of the depth distribution of the microbial community off Chile and that of the principal shrimp (both penaeid and galatheid) and hake fishing grounds[9], strongly suggests a possible trophic relationship in view of the fact that most of the standing-crop therein seems to be made up of filamentous bacteria.

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Drinking behaviour induced by intracranial injections of eledoisin and substance P in the pigeon

THE only peptide known which rapidly and consistently elicits a specific behavioural response when injected into the brain is angiotensin II, which in picomole doses causes reptiles, birds and mammals to seek out and drink water[1]. But, there is evidence to suggest that other peptides such as substance P may also participate in the control of brain function and behaviour. Substance P is distributed throughout the mammalian peripheral and central nervous system, particularly in myenteric plexus, sensory afferent pathways, hypothalamus and substantia nigra[2]. In the human brain regional concentrations as high as 0.5×10^{-9} mol per g wet tissue have been reported[3]. Substance P is a member of a family of peptides with similar pharmacological properties and which also includes eledoisin, an undecapeptide obtained from the salivary glands of certain Mediterranean cephalopods[4]. These peptides share a similar amino acid sequence at the C-terminal end (Fig. 1). Both eledoisin and substance P stimulate nerve cell activity in vertebrates and invertebrates and may act as regulators of neural activity[5], but no specific behavioural effect of these peptides has yet been reported. We report here that eledoisin and substance P elicit vigorous drinking when injected into the brain of conscious pigeons.

At a preliminary operation intracranial cannulae (outside diameter (o.d.) 0.6 mm) were implanted stereotaxically in male and female pigeons (*Columba livia*, 300–450 g) under Equithesin anaesthesia (3.0 ml per kg body weight). The cannulae terminated in the lateral or third ventricle, preoptic area, lateral hypothalamus or striatum. The pigeons were allowed at least one week to recover from the operation before testing began. The injections were made in conscious birds through stainless steel tubing (o.d. 0.3 mm) inserted into the implanted cannulae and projecting 1 mm beyond the cannula tip into the brain tissue. All drugs were dissolved in 0.9% saline and injected in volumes of 1 μl over 10 s. The highest dose of eledoisin was injected in 10 μl because of its low solubility. The pigeons were returned to their home cages immediately after injection where food, water and mineral grit were available. Only one drug was tested per day, with at least a 2-d rest between tests. The order of testing was random. The drugs injected were eledoisin (Farmitalia), eledoisin-related peptide, substance P, Asp^{1}Ile^5-angiotensin II, arginine vasotocin (all from Bachem), vasopressin (Pitressin, Parke-Davis), bradykinin (Sigma) and L-glutamate (Sigma).

Intracranial injections of as little as 10^{-11} mol eledoisin consistently caused pigeons to drink water. The drinking was vigorous but completely normal, and usually began within 1 or 2 min after injection. The mean latency ($\pm$s.e.m.) in 13 pigeons in response to 10^{-10} mol of eledoisin was 1.5 ± 0.2 min. The amount of water drunk was dose dependent and was large (Fig. 2). Intracranial injection of 10^{-9} mol eledoisin caused pigeons to drink more than they would normally ingest during the entire day, usually in a period of less than 5 min. The response was repeatable from day to day and always specific to drinking. None of the birds ever ate or engaged in any other behaviour consistently after injection. When the water spouts were removed from the cage the pigeons repeatedly looked around the cage for water and pecked frequently at the hole where the water spout normally projected. Immediately the spout was replaced they drank vigorously. When drinking was prevented eledoisin did not cause increased body water losses, as measured by changes in body weight, and there were no changes in cloacal temperature. Intravenous injections of 10^{-10} or 10^{-9} mol of eledoisin did not cause pigeons to drink ($N = 5$), indicating that the effect of intracranial injections was not due to leakage from the brain.

Since this drinking response was identical to that which we observed following intracranial injections of angiotensin II in the pigeon[6], we compared the relative potencies of these two peptides. On a molecule per molecule basis eledoisin was only about one order of magnitude less potent than angiotensin II (Fig. 2). Thus in the pigeon eledoisin is as active a dipsogen as carbachol is in the rat[1].

The C-terminal hexapeptide fragment of eledoisin (eledoisin-related peptide) and substance P also caused pigeons to drink when injected into the brain. These peptides were less active than eledoisin, but again the response was specific to drinking and the amount of water drunk was significantly greater than that following control injections of 0.9% saline (Fig. 2). On the other hand, intracranial injections of vasopressin (10^{-12},10^{-11} mol), vasotocin (10^{-11},10^{-9} mol), bradykinin (10^{-10},10^{-9} mol) or L-glutamate (10^{-10},10^{-7} mol) had no significant effects.

Eledoisin caused drinking in all brain regions sensitive to the dipsogenic actions of angiotensin II. These included the lateral ventricle ($N = 3$), third ventricle ($N = 3$), preoptic region ($N = 6$) and lateral hypothalamus ($N = 4$). There were no significant differences in the response to eledoisin from these four areas but intakes tended to be highest following preoptic or third ventricular injection. Two pigeons with cannulae in the neostriatum which did not pass through the lateral ventricle failed to drink to

Fig. 1 Amino acid sequences of peptides that stimulate drinking when injected into the brain of the pigeon.

Angiotensin II	Asp - Arg - Val - Tyr - Ile - His - Pro - Phe
Eledoisin	Pyr - Pro - Ser - Lys - Asp - Ala - Phe - Ile - Gly - Leu - Met (NH$_2$)
Eledoisin Related Peptide	H$_2$N Lys - Phe - Ile - Gly - Leu - Met (NH$_2$)
Substance P	Arg - Pro - Lys - Pro - Gln - Gln - Phe - Phe - Gly - Leu - Met (NH$_2$)

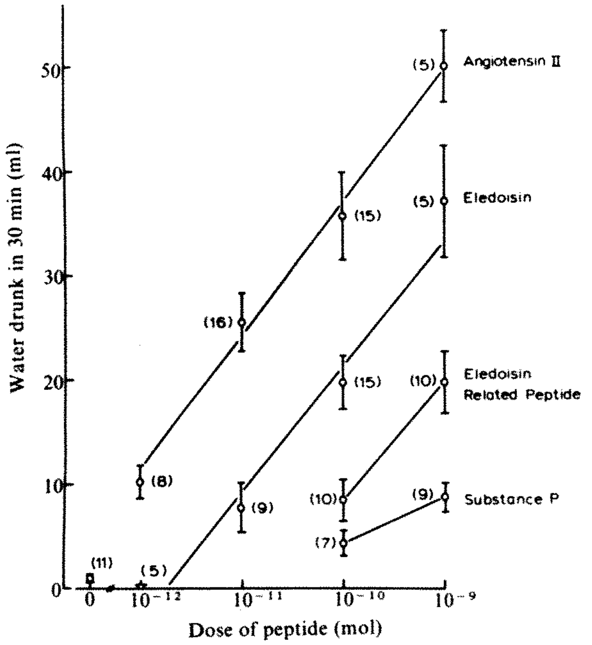

Fig. 2 Mean amounts (±s.e.m.) of water drunk in 30 min (no. of birds tested in parentheses) following intracranial injection of different peptides. Controls received 1 μl 0.9% NaCl. Regression lines are shown for angiotensin II and eledoisin.

eledoisin or angiotensin II. One pigeon with a striatal cannula which did pass through the lateral ventricle drank to injections of the peptides, probably because of reflux of the injected material into the ventricular system as has been shown for angiotensin II injections in the rat[7].

The similar time course and dose-response relationship of drinking following intracranial injections of angiotensin and eledoisin in the pigeon and the similar neuroanatomical distribution of sensitive brain sites suggest a similar or common mechanism. It is unlikely, however, that these two peptides are activating the same receptor site, because eledoisin is structurally so very different from angiotensin II and because we have found that the receptor sites for angiotensin-induced drinking in rats and pigeons are highly specific for the angiotensin II octapeptide. Only slight modifications or removal of amino acids in the angiotensin peptide chain, particularly at the C-terminal end, severely reduce or even abolish activity altogether[8,9]. Other peptides such as vasopressin, vasotocin or bradykinin all fail to stimulate drinking in the pigeon.

Eledoisin and substance P are potent stimulants of neuronal activity[10], but a nonspecific activation of nervous tissue cannot explain the consistent and specific behavioural response observed here. Drinking was elicited by injection of these substances into several different brain regions which also contain the neural elements of many other behavioural functions. Furthermore, intracranial injections of glutamate, another potent excitatory substance, did not cause pigeons to drink.

This is the first demonstration that eledoisin and substance P can elicit a specific and complete behavioural response when injected into the brain. What is surprising is that this response should be identical to that elicited by angiotensin II, the only other peptide known to have such a pronounced behavioural effect. Angiotensin II is a potent vertebrate hormone that is released in response to extracellular fluid losses and has many actions which contribute to the restoration of body fluid balance[11]. The significance of the drinking response to eledoisin and substance P in terms of body fluid regulation remains to be determined.

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Cells selective to binocular disparity in the cortex of newborn lambs

In the nineteenth century there was a vigorous controversy over the role of visual experience in the development of stereoscopic depth perception[1,2]. Recent neurophysiological findings suggest that certain cells in the visual cortex of cats[3,4], monkeys[5] and sheep[6] may provide the neural basis for stereopsis. Each cell is driven optimally by a stimulus at a certain distance from the animal, and different cells prefer different retinal disparities corresponding to objects at various distances. In cats, the neural pathways from the two eyes to individual cortical cells are labile and may be modified by visual deprivation during the first few weeks of life[7–10]. The visual cortex of the newborn kitten is immature and disparity selectivity appears only after exposure to a normal visual environment[11]. We find, however, that neither of these two conclusions is true of lambs.

It has been suggested that the neural mechanisms underlying stereopsis require visual experience for their development[11–15]. 'Plasticity' of binocular correspondence was observed by Schlaer[16] and was thought to account for the preservation of correspondence in spite of changing interocular geometry during development[14]. Newborn animals (including lambs) will avoid a 'visual cliff'[17,18], but it is unclear whether stereopsis is involved, particularly since chicks and rats will continue to avoid the cliff even with one eye closed[17]. Good depth judgment would be of obvious survival value in the wild, where a 1-d-old mountain lamb will avoid a ravine[19].

We have begun to investigate the possible role of visual experience in the development of stereopsis by recording from the visual cortex of lambs. In adult sheep (and goats) a substantial number of cells, especially in V_2, can be driven only by a binocular stimulus at a particular disparity. In V_2, the optimal vertical disparities are close to zero but a wide range of horizontal disparities (about ±4°) occurs[6]. Our strategy was to look for such depth-sensitive cells in the visual cortex of newborn lambs which have had no visual experience. Lambs are born robust and with clear optic media, which makes them very suitable for studying the development of disparity detecting mechanisms, especially since large horizontal disparities occur in the adult.

Six animals (five newborn lambs and one newborn kid) were used. Three were born and kept in complete darkness, and recorded from on the first, fifth and sixth days after birth. The other three were born in the light (albeit moonlight), kept in normal lighting, and recorded from within 21 h of birth. They were anaesthetised initially with 2% halothane in oxygen and later maintained on intravenous pentobarbitone (0.5 mg per kg body weight per h) and a N_2O/O_2 mixture (60%, 40%). This dosage was sufficient to give full surgical anaesthesia in newborn lambs, and larger doses caused respiratory arrest. Tubocurarine (0.1 mg per kg per h) and gallamine triethiodide (3 mg per kg per h) were used as paralysants to reduce eye movements. (For details see ref. 6.)

Tungsten-in-glass microelectrodes with impedances of 1–5 MΩ at 1,000 Hz were used[20]. Visual stimuli were usually hand-held targets moved against a screen 90 cm from the eyes and the separate monocular receptive fields were drawn on a tangent screen. For most of the units we also tested the effect of varying the retinal disparity by means of a Risley prism in front of the left eye. Units and background activity were tested once every 100 μM or so of electrode descent.

The positions of the eyes were monitored using a Fison indirect ophthalmoscope. The projections of the optic disk and vertical retinal artery were plotted on extensions of the tangent screen. Hence eye movements greater than 0.5° could be detected and corrected for.

Although the visual mapping on the cortex sufficed to distinguish V_1 and V_2, we identified the needle tracks histologically. Of the 231 units whose responses we tested, 30 were in V_1, 192 were in V_2, and 9 were not reliably located : 119 of the V_2 units were from lambs with no visual experience whatsoever. Here we describe only the units in V_2.

The results were clear-cut. Almost all the units were visually responsive, and more than 95% were sharply tuned to orientation. There was no noticeable difference in the proportion of tuned cells or in their sharpness of tuning compared with the adult. Most of the units responded much better to a moving oriented bar than to a moving spot and those which would respond to a flashed bar were selective to its orientation. In this sense they were truly orientation selective rather than direction selective cells. Also the cells seemed to be arranged in 'orientation columns' and systematic changes in orientation could be seen as the electrode crossed several columns (compare with baby monkey[22]). Figure 1 illustrates one such 'orientation sequence' from the very first penetration made in one of our newborn lambs. The orientations of the receptive fields of individual binocular neurones were determined separately through each eye, and were found to be precisely matched. Thus, in lambs, at least, there is no basis for the suggestion that the alignment of the optimal orientations in the two eyes requires visual experience[15,21].

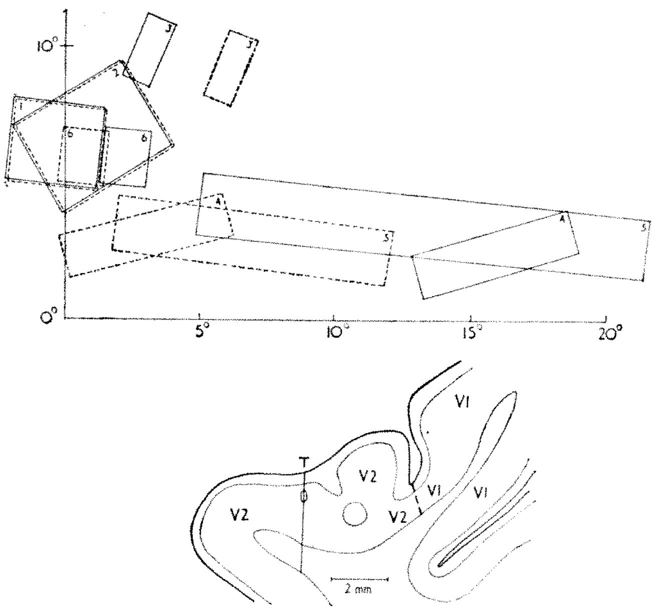
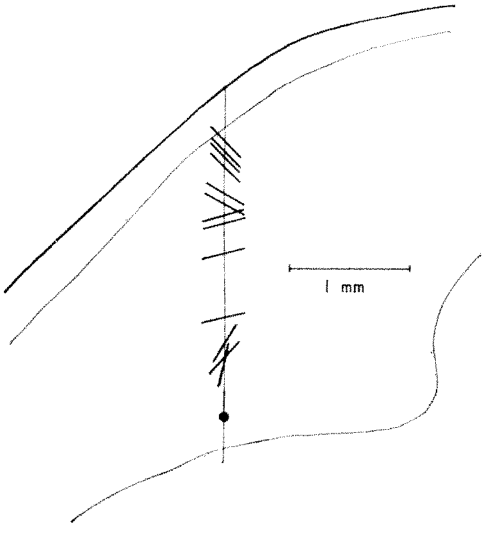


Fig. 2 The monocular receptive fields, through each eye, of the single units recorded in a penetration through V2, illustrating the occurrence of binocular disparities. This particular lamb was born 21 h before the start of the experiment, but it had less than 12 h of visual experience. Comparable results were obtained in a lamb with no visual experience whatsoever. The zero vertical meridia of the two eyes have been aligned, so the receptive fields of zero disparity cells are exactly superposed. The fields of the left eye are shown by discontinuous lines, and the numbers indicate the order in which the units were recorded. It can be seen that the disparities were purely horizontal and spanned a very large range on account of two units with unusually large (up to 13°) uncrossed disparities. We confirmed these disparities by tracking back and down again with the electrode, and we checked the eye positions after each large change of disparity. Also, the orientations of the receptive fields are the same for the two eyes. The inset shows the position of the electrode track (T), including a lesion, in V2. The V1/V2 border has been estimated by comparing the section with one 1 mm posterior in which the site where the visual map reversed was found with five electrode penetration.

Fig. 1 An orientation sequence. The optimal orientations of 13 single units are plotted at the depth they were recorded in a penetration through V2. The only other unit recorded was not clearly orientated, and its position is marked by a filled circle. Clearly, the optimal orientation changed monotonically as the electrode advanced through the cortex, which suggests that the systematic organisation of orientation columns which occurs in the adult was already present. This lamb was born in complete darkness a few hours before the experiment, and its eyes were first exposed to light a few minutes before the start of this, the first penetration.



The receptive field properties did not differ noticeably from those in adults, and simple, complex and hypercomplex cells could all be found (as in the cortex of newborn monkeys[22]). There was one exception, however: 16 units were found for which the response was excitatory from one eye and strongly inhibitory from the other, with greatest inhibition at zero disparity. We have not so far found such units in adult sheep. The separate monocular fields of binocular neurones could be mapped on the tangent screen, and variations in binocular disparity (that is, the range of 'scatter' of receptive field disparities) could be precisely determined (Fig. 2). The most frequent disparity was taken to be zero. In three of the animals there was clear evidence of horizontal disparities of up to ±4°, but in one animal there were two cells with exceptionally large disparities (Fig. 2). The range of vertical disparities was close to zero in all six animals. Disparity 'tuning' curves could be obtained for many of the cells by varying the strength of the prism in front of the left eye. The peak of the tuning curve usually corresponded to what one would expect from actually measuring the angular distance between the centres of the monocular receptive fields. Hence cells selectively 'tuned' to a large range of non-zero disparities, as well as zero, were observed. These results cannot have been seriously contaminated by eye movements, since the positions of the eyes were monitored to within 0.5°, and the disparities were much larger than this. Furthermore, on some occasions we withdrew the electrode and drove it down the track again, and the same disparities were found at the same depths.

There were interesting differences between the V_2 binocular neurones of newborn lambs and those seen in the adults (more than three months). In adults most of the neurones give a strongly facilitated response to a binocular stimulus at the optimal disparity and many are true 'and-gates', not having discernable monocular receptive fields. In newborn lambs, however, *all* the binocular neurones could be independently driven through either eye and the degree of binocular facilitation or 'synergy' was considerably less than in the adult. The binocular facilitation in a normal seven-week-old lamb in which we recorded from 36 units was intermediate between that in the adult and in the newborn lamb. Whether the conversion of immature binocular neurones into true 'and-gates' requires visual experience, remains to be determined. The appearance of additional inhibitory mechanisms may be required for this.

In visually naive kittens, Pettigrew[11] found that the neurones were not selective to disparity and yet showed binocular facilitation at least as great as in the adult. This is the opposite of what we have found in lambs, where many of the cells were tuned to disparity, but the binocular enhancement was substantially less than in the adult. While several authors[9,11,14-16] have suggested that visual experience may have an active role in organising the feature-detecting properties of cortical neurones, there seems little need for this in the lamb, for we find that in virtually all the cells in V_2, the following are all specified by a genetic mechanism: (1) orientation selectivity; (2) organised 'orientation columns' and systematic changes in orientation tuning (orientation 'sequences'); (3) interocular matching of orientation selectivity, and (4) 'hypercomplex' type receptive field organisation. Most importantly, we have found that disparity selectivity is present at birth. This confirms Hering's theory[1] that the establishment of correspondence between disparate retinal areas does not require visual experience, although the progressive increase in the degree of binocular facilitation may turn out to require visual experience.

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Early hyperthyroidism alters the distribution of mossy fibres in the rat hippocampus

Daily injections of thyroid hormone initiated on the day of birth and continued for several weeks, significantly accelerate the differentiation of cerebellar granule cells[1-3], which are formed after birth[4]. Since most of the hippocampal granule cells are also generated postnatally, early hyperthyroidism was chosen as a means of studying possible mechanisms involved in the formation of synapses between granule cell axons (mossy fibres) and pyramidal cell dendrites. This note provides the light microscopic demonstration, with the Timm silver sulphide method[5], that hyperthyroidism in the rat consistently results in the formation of ectopic hippocampal mossy fibres. Electron microscopic and Golgi studies are in progress and will be published separately.

Basic mechanisms governing the formation of neuronal circuitries are as yet poorly understood. The spatial segregation of afferents along defined regions of the postsynaptic surface in neurones such as the cerebellar Purkinje cells and hippocampal pyramidal and granule cells indicate a high degree of specificity during neural ontogeny. At present, it is unclear how intrinsic neuronal properties, temporal factors, specific affinities and recognition processes interact in such development. The strict segregation of afferents along the pyramidal cell surface, in the hippocampus, first clearly conceived by Cajal[6] and further explored by Lorente de No[7], as well as the unique synaptic relationship between mossy fibres and the large protuberances (or 'excrescences') on the apical dendritic shaft of the pyramidal cell, make this an excellent region to study synaptogenesis, especially the relative parts played by the pre- and postsynaptic elements[8]. Genetically associated variations of this system have recently been reported in the mouse[9].

Since the synaptic boutons of the mossy fibres contain a metal (most likely zinc)[10], which can be stained histochemically using the Timm silver sulphide procedure[5], an easy and specific bulk method is available for light microscopic screening of large numbers of sections with respect to alterations in the distribution of granule cell axons. Hooded rats (males, 8 per litter) were made hyperthyroid by the subcutaneous injection of daily doses of 5–15 μg L-thyroxine in 0.1 ml physiological saline from the day of birth until day 15. Control animals were injected daily with 0.1 ml physiological saline during the same period. All animals were killed 30 d postnatally, by cardiac perfusion with 1.19% sodium sulphide and 5% polyvinyl pyrrolidone (R/P) in 1 l of 60% ethanol, preceded by a short prewash with physiological saline under choral hydrate anaesthesia. Brains were dehydrated, embedded in Paraplast, and sectioned serially along the horizontal plane. Slides were stained using Timm the silver sulphide method for the histochemical visualisation of mossy fibres as described extensively by others[5,9,11]. A total of eight hyperthyroid and eight control animals were used in this study, but more than 20 hyperthyroid animals have been examined, when rats injected with thyroxine according to different schedules are included.

In controls, as in the normal albino rat, the mossy fibres pass into the hilus of the fascia dentata, then course along the pyramidal cell layer largely transverse to the septotemporal axis of the hippocampal formation[7,12,13]. Most of these axons synapse *en passant* with the excrescences on the apical dendrites of pyramidal cells. These fibres form the suprapyramidal

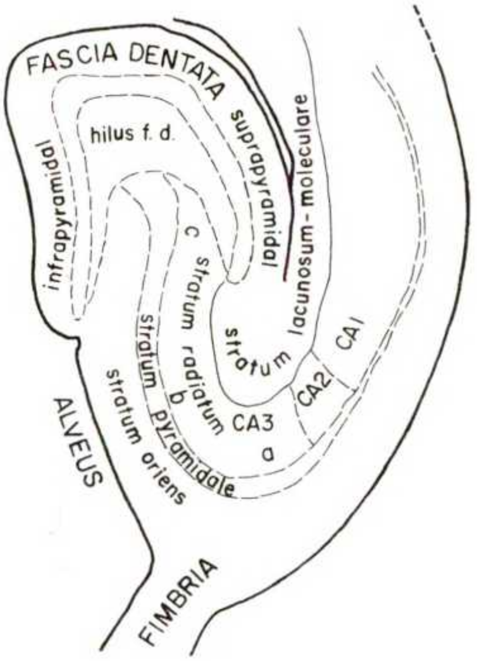

Fig. 1 Diagram of Ammon's horn in the horizontal plane to indicate nomenclature. Regio inferior comprises CA3 and CA2; Regio superior, CA1. Although it is unclear whether CA2 exists as a defined area and to what extent its pyramidal cells receive mossy fibre terminals[13,15], we have designated with this term the border zone between Regio inferior and Regio superior within which the suprapyramidal mossy fibre bundle terminates, in accordance with the terminology used by Chronister and White[15], Angevine[19] and Hine and Das[21].

mossy fibre bundle, extending as far as the border between Regio inferior (CA3–CA2) and Regio superior (CA1) (see terminology Fig. 1). Others take a more ventral, intra- and infrapyramidal route for a short distance, synapsing with excrescences of both the basal and apical dendrites of pyramidal cells in subfield CA3$_c$ before joining the suprapyramidal bundle (Fig. 2*a*)[13-15].

At most ventral and dorsal levels (not illustrated) some infrapyramidal mossy fibre terminals are also present in CA2 and CA3$_{a-b}$. It is known, however, that at the middle septotemporal level, as defined by Haug[11], the infrapyramidal zone in CA2 and CA3$_{a-b}$ contains few, if any, mossy fibre terminals (Fig. 2*a*). It is at this level of the hippocampus that we found the most clear-cut changes in the experimental animals. As shown in Fig. 2*b*, at this level in the hyperthyroid rat a dense layer of Timm reaction product, whose thickness is approximately one-third of the suprapyramidal mossy fibre layer, is consistently located in an infrapyramidal position in CA3$_{a-b}$, and CA2, reaching the border with CA1. Rows of Timm granules cross the pyramidal cell layer, suggesting that many infrapyramidal mossy fibres originate from the suprapyramidal bundle. A number of Golgi rapid sections available now supprt the former view, although it is also possible that some fibres originate from the infrapyramidal bundle. Also, preliminary electron microscopic studies show bundles of infrapyramidal mossy fibres in hyperthyroid rats with *en passant* mossy swellings synapsing on excrescences of the basal dendritic stems of pyramidal cells in CA3$_{a-b}$. In normal animals the basal dendrites of pyramidal calls receive little, if any, mossy fibre input in this field of Ammon's Horn, and have smooth shafts with few and simple spinous evaginations.

Why the lower border of the mossy fibre bundle is drastically changed and the transverse (between Regio inferior and Regio superior) and upper (towards the stratum radiatum) borders are not, may depend on the time at which the administered hormone starts to interfere with the morphogenetic process.

Various studies on hippocampal development [16-23] indicate that in addition to specific chemical affinities and recognition phenomena, temporal factors are of considerable morphogenetic importance. Complex spatio–temporal relationships between the time of origin and subsequent differentiation of granule cells and pyramidal cells have been demonstrated. These patterns include the start of granule cell differentiation in the suprapyramidal (lateral) blade of the dentate gyrus earlier than in the infrapyramidal (medial) blade and the birth of granule cells nearest the molecular layer before those towards the hilus[16,17] an 'inside-out' pattern of pyramidal cell generation[19,21] in that the deep pyramidal cells are born before those more superficial in the pyramidal layer, and the formation of apical pyramidal cell dendites earlier than basal dendrites[22]. Such spatio–temporal relationships between the differentiation of hippocampal granule cells and their pyramidal cell targets could provide an ideal system for the formation of discrete mossy fibre pyramidal cell connections, as suggested by recent studies in the mutant mouse[23].

How the position of individual mossy fibres within the suprapyramidal bundle is determined remains to be ascertained. In the cerebellum the parallel fibre growth is appositional, that is, the lower fibres are formed first and the most superficial last[24,25]. The existence of a chronological-positional order in

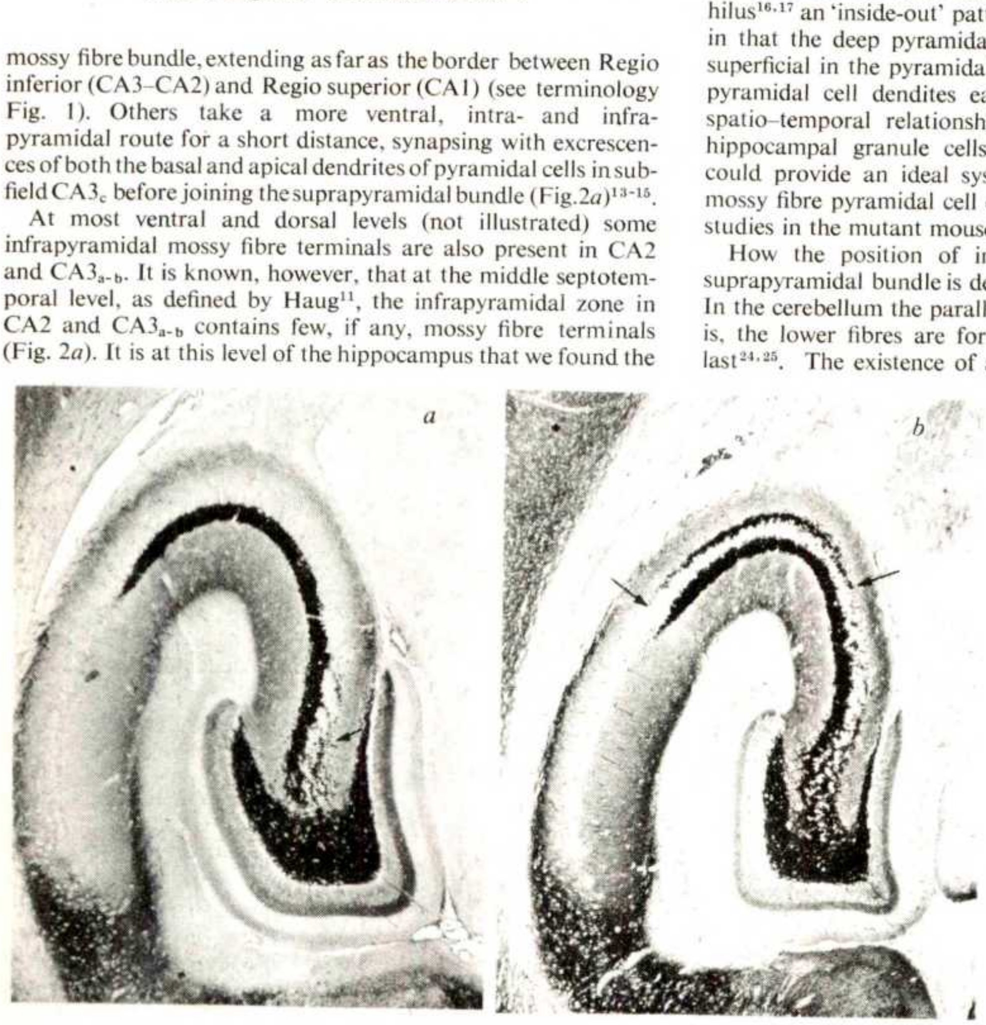

Fig. 2 *a*, Horizontal hippocampal section from saline-injected 30-d-old rat; Timm staining method; middle septotemporal level of the hippocampus as defined by Haug[11] (his Fig. 2*c*). The Timm precipitate marks the hilus fascia dentatae, the mossy fibre layer in CA3$_{abs}$ and that part of CA3$_c$ where mossy fibre bundles run through the pyramidal layer reaching an infrapyramidal position (arrow). In CA2 and especially CA3$_{a-b}$ the infrapyramidal zone shows only scattered Timm-positive granules (unlabelled). ×30.2 *b*, Horizontal hippocampal section from a 30-d-old rat injected daily with 10 µg L-thyroxine from birth to day 15. Middle septotemporal level of the hippocampus as defined by Haug[11]. Note the conspicuous Timm staining in the infrapyramidal zone of CA2 and CAC$_{a-b}$ (arrows). ×30.2

the hippocampal mossy fibre bundle is suggested by the fact that with postnatal hyperthyroidism the ectopic synapses are formed infra- and not suprapyramidally.

Other available data suggest that the abnormally expanded mossy fibre area in the hyperthyroid rat may be a consequence of an exaggerated growth of postnatally-generated granule cell axons and of an acceleration of granule cell differentiation. An effect of thyroid hormone on axonal growth has been indicated in recent developmental and regeneration studies[1,26]. Moreover, in the cerebellum, early hyperthyroidism accelerates the peak of postnatal granule cell differentiation[2]. This experimental condition also results in a permanent deficit in total cell number in the cerebrum and cerebellum[27], apparently caused mainly by early cessation of proliferation of germinal cells which results in a smaller number of postnatally generated microneurones. It would be interesting to know whether the number of hippocampal granule cells is also reduced. The effect of thyroid hormone on growth of the hippocampal mossy fibres could be more powerful than it seems from our work if the expanded mossy fibre area were produced by a smaller number of granule cells.

Although a primary effect on the presynaptic element seems probable, at present neither a direct influence of thyroid hormone on the pyramidal neurones, nor an indirect one by way of other hormones can be ruled out, especially since the dendritic tree of the pyramidal neurones is still undergoing development during the hormonal treatment. The aberrant mossy fibre terminals may occupy vacant postsynaptic sites, successfully compete for existent synaptic loci, or induce the formation of new ones. These questions and whether the ectopic mossy fibres are functionally active, remain to be determined.

Our results are somewhat germane to the demonstration of axonal 'sprouting' and 'expansion' of undamaged terminal areas after partial de-afferentation of various cortical and subcortical areas during development[28]. The hormonal treatment, however, affords the possibility of producing heterotopic synapses without the degeneration of neuronal elements and the glial cell proliferation which follows de-afferentation. The expanded target area of the mossy fibres in the hyperthyroid rat, represented by the accessory infrapyramidal bundle in CA2 and $\mathrm{CA3}_{\mathrm{a-b}}$, indicates that the normal pattern of axonal growth can be altered experimentally at a site where segregation of afferents appeared so strict as to be paradigmatic.

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Generality of oestrogen stimulation of peroxidase activity in growth responsive tissues

OVER 20 years ago, Stotz *et al.*[1] reported that administration of oestrogen to rats effected an increase in the peroxidase (EC 1. 11. 1. 7) activity of the uterus. Subsequently, this stimulation of uterine peroxidase activity was attributed to an influx of eosinophils into the tissue[2]. More recent evidence, both biochemical[3–5] and histochemical[6–7], clearly indicates a uterine origin for the enzyme. On the basis of histochemical peroxidase assays, we proposed that peroxidase may be a useful marker protein response elicited by oestrogen in those rat tissues, like uterus[8], vagina[8] and mammary tumour[9], whose growth is regulated by oestrogen. Recent data[10] on the dose and steroid dependence of rat uterine peroxidase induction also supports this proposition. To be a meaningful marker for the effect of oestrogen on growth responsive tissues, the peroxidase induction should be demonstrable in animals other than the rat and should show a reasonable tissue specificity. This communication reports on the generality and specificity of oestrogen regulation of peroxidase activity.

Rats (Sprague–Dawley strain), mice (Swiss Albino strain), and hamsters (Golden Syrian strain) were obtained from ARS/Sprague–Dawley, Madison, Wisconsin. Guinea pigs (Hartley strain) were obtained from Camm Research, Wayne, NJ. Animals were injected with oestradiol-17β, (Sigma) 40 μg per kg body weight in 25% ethanol–saline or vehicle alone, 44 and 20 h before decapitation. Trimmed tissues were homogenised in cold 10 mM Tris-HCl, *p*H 7.2 buffer, 25–50 mg tissue per ml, centrifuged 45 min at 39,000*g* 2 °C and the peroxidase activity solubilised by rehomogenising the sediments with 0.5 M $CaCl_2$ in 10 mM Tris buffer, *p*H 7.2. The extracts were collected after 45 min, 39,000*g* centrifugation at 2 °C. Although both the hypotonic and $CaCl_2$ extracts were assayed for peroxidase activity, only minimal activity was found in the original 10 mM Tris supernatants (<5% of total).

Peroxidase assays were performed by modification of the method of Himmelhoch *et al.*[11], at 25 °C. The assay mixture, 3.0 ml total volume, contained 13 mM guaiacol and 0.3 mM H_2O_2 in 10 mM Tris buffer, *p*H 7.2 containing 0.5 M $CaCl_2$. The reaction was started by the addition of enzyme (extract) and initial rates of guaiacol oxidation were determined from the increase in absorbance at 470 nm for the first minute. An enzyme unit was defined as the amount of enzyme required to produce an increase of one absorbance unit per minute in these assay conditions.

Table 1 shows the effect of two injections of oestradiol on the uterine wet weight and uterine peroxidase activity in four species of laboratory animals. In each case, there was a significant increase in both uterine weight and uterine peroxidase content but it is apparent that there were appreciable differences in the uterine sensitivity of these various animals to oestrogen, given at identical doses based on body weight. However, the relative increases in peroxidase content closely parallel the relative increases in weight of the uteri. Thus, the hamster showed the smallest increase in uterine weight (35%) and the smallest

Table 1 Oestradiol stimulation of the uterus of various species

Animal (age/wt)	Group*	Uterine wt (mg)	Uterine peroxidase activity Units (per g ± s.e.m.)	Uterine peroxidase activity mUnits per uterus
Hamster	C	45.8	0.064±0.018	2.9
(25 d/46 g)	E	62.4	0.132±0.016	8.2
	(E/C)	(1.36)	(2.06)	(2.9)
Guinea pig	C	232	0.18±0.04	42
(23 d/210 g)	E	534	0.83±0.17	443
	(E/C)	(2.30)	(4.61)	(10.5)
Rat	C	25.2	0.27±0.09	6.8
(30 d/54 g)	E	84.3	24.6±7.3	2074
	(E/C)	(3.35)	(91.1)	(305)
Mouse	C	7.8	0.1±0.04	0.78
(28 d/19 g)	E	35.0	29.3±10	1,026
	(E/C)	(4.49)	(293)	(1,315)

*Animals (4–10 per group) received subcutaneous injections of vehicle(C) or oestradiol (E) 40 μg kg^{-1} body weight, 44 and 20 h before killing. E/C is ratio of weight or peroxidase of oestrogen treated to control uteri.

increase in uterine peroxidase (2-fold). The most dramatic uterine sensitivity to oestradiol was seen in the mouse in which the uterus grew about 4.5-fold and showed almost a 300-fold increase in peroxidase concentration. The rat and mouse showed remarkable (300- and 1,300-fold) increases of total uterine content of peroxidase in 44 h of oestradiol treatment.

The oestrogen stimulation of peroxidase activity seems to be restricted to growth-responsive tissues (Table 2). Significant increases in peroxidase concentration are seen in both the uterus and vagina of the immature rat given oestradiol, but no peroxidase is found in the pituitary. Although small to large concentrations of peroxidase can be found in the kidney, thyroid, lung and spleen, no appreciable change in the peroxidase content of these tissues occurs on oestradiol treatment. Other tissues assayed, specifically ovary, adrenal and liver, had no significant peroxidase in the oestrogen-treated or untreated rat. Although the peroxidase content of the thyroid of the immature animal is low, the adult rat thyroid peroxidase content (about 40 units g^{-1}) was not influenced by oestrogen treatment.

Figure 1 shows the peroxidase content of some human oestrogen-responsive tissues. The endometrium and myometrium were obtained by physical separation from small samples of human uterus. While care was taken to obtain pure endometrium, it is probable that the myometrium contained some residual glandular tissue. Clearly, the peroxidase content of uterine endometrium exceeds that of the myometrium. Samples of human breast cancer tissue showed a wide range of peroxidase content. Nine of the 39 samples (23%) had no peroxidase, while 11 (28%) had an appreciable amount, that is greater than 1 unit g^{-1}.

Up to the present, there has not seemed to be any particularly meaningful protein marker for oestrogen action in the mammalian uterus. Although the induced protein or IP, first described by Gorski *et al.*[12] has generated considerable interest in this regard, a recent report[13] suggests that this protein may be limited to the rat uterus; it was not found in the uterus of the mouse, rabbit, hamster or guinea pig, or in the rat vagina. Whereas certain enzymes, especially those involved with carbohydrate metabolism, have been found to increase in the uterus of the oestrogen treated animal, the extent of these increases are generally small, at best 5–10-fold[14], and largely reflect the increased metabolic activity of the tissue.

Table 2 Tissue specificity of oestrogen effect on peroxidase activity

Rat tissue	Peroxidase activity (units per g) Control	Peroxidase activity (units per g) E2 treated*
Uterus	0.16	32.4
Vagina	0.0	5.8
Pituitary	0.0	0.0
Kidney	0.4	0.5
Thyroid	0.4	0.5
Lung	15.6	20.4
Spleen	186	188

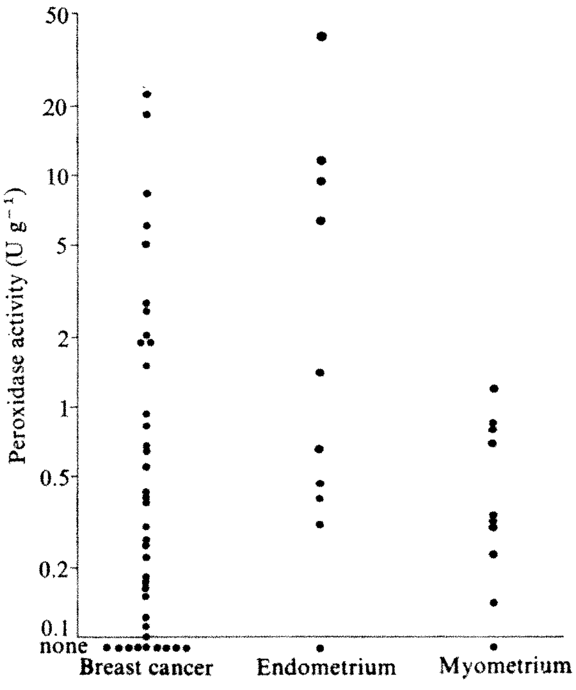
*Immature female rats given oestradiol-17β, 40 μg per kg body wt, subcutaneously 44 and 20 h before killing.

Uterine peroxidase activity seems to be a good marker for oestrogen stimulation of growth. As shown in this report, oestrogen effects a growth-related increase in peroxidase concentration in the uterus of various species. Furthermore, the oestradiol stimulation of the uterine peroxidase content of the immature mouse and rat uterus, as much as a 1,000-fold increase, is certainly one of the most dramatic effects of oestrogen. Peroxidase stimulation by oestrogen is limited to the growth-responsive normal tissues, uterus and vagina, and is not seen in the pituitary where the response to oestrogen is largely functional. Oestrogen-independent tissues of the rat show no significant stimulation of peroxidase content, whether or not the tissue of the untreated animal contained any enzyme.

An earlier report[15] indicated that the peroxidase content of human endometrial brushings showed a variation coinciding with the menstrual cycle. Since the human uterine samples reported here were from pre-menopausal patients, the range of peroxidase content probably reflects menstrual cycle variation. The high endometrial peroxidase concentrations agree quantitatively with the concentrations found in the oestrogen-stimulated rodent uterus (Fig. 1 and Table 1), suggesting that studies on the regulation of peroxidase in the rodent uterus are quite relevant to the human.

It is particularly interesting to find that a significant

Fig. 1 Peroxidase content of human uterus and breast cancer. Calcium chloride extracts of samples of human endometrium, myometrium and breast cancer tissue assayed for peroxidase content.



number of human breast cancer specimens contain appreciable amounts of peroxidase activity. An earlier report from our laboratory described the oestrogen regulation of peroxidase in the carcinogen-induced mammary tumours of the rat[9]. Data of the last 5 yr have indicated that essentially only those patients whose breast cancer tissue contains appreciable amounts of oestrogen receptor respond to endocrine therapies[16,17]. Since, however, at least a third of the patients with oestrogen receptor positive tumours do not respond, it is apparent that the presence of oestrogen receptor is an essential, but not necessarily sufficient, condition for response. It is possible that use of an assay of a biochemical parameter, possibly peroxidase, which reflects tissue hormone-dependence may enhance the accuracy of prediction of response in those breast cancer patients whose tumours contain oestrogen receptor. Such studies are in progress.

The generality and specificity of the oestrogen stimulation of peroxidase activity in tissues which are stimulated to grow by oestrogen recommends this marker as a meaningful parameter for the more detailed studies of steroid hormone regulation of gene function as related to growth. Further study of peroxidase content of normal and neoplastic human tissues can provide a better understanding of hormonal regulation of these tissues and possibly a more accurate diagnosis of oestrogen-dependent neoplasms.

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Comparative drug sensitivities of culture forms of *Trypanosoma cruzi* and *Trypanosoma dionisii*

AT least 12 million people suffer from Chagas' disease in South America, but despite intensive efforts over the last 65 yr, no treatment effective against all stages of the disease has emerged[1]. *Trypanosoma cruzi* the causative agent of the disease, develops intracellularly in the tissues of the heart and alimentary tract. Such development, together with the absence of effective chemotherapy, means that drug testing for agents active against Chagas' disease is time-consuming, costly and hazardous[2]. Because of the danger of infection when working with *T. cruzi*, it has been suggested by Baker[3] that other species of the subgenus *Schizotrypanum* of the genus *Trypanosoma* could be used as models for studying this parasite. *T. dionisii*, isolated from the bat, *Pipistrellus pipistrellus*, by Baker and Thompson[4], seems to be a particularly suitable choice because of its apparent non-pathogenicity in man[3]. The experiments described here show that *T. dionisii* can replace *T. cruzi* in *in vitro* drug screens.

Table 1 Effects of drugs known to be active in animal screens on the culture epimastigotes of *T. dionisii* and *T. cruzi*

Drug* (µg ml^{-1})		Growth (% of control growth) *T. dionisii* 24 h	72 h	*T. cruzi* 24 h	72 h
Bayer 7602 Ac (bisquinaldine)	100	98	100	102	99
Spirotrypan (arsenobenzene)	1	113	120	98	103
	10	12	18	2	1
	100	0	0	0	0
Ethidium Br (phenanthridine)	1	58	41	97	98
	10	59	10	56	15†
	100	26	5	9	5†
Pentaquine (8-aminoquinoline) diphosphate	1	93	91	27	21
	10	46	5	25	7
	100	76†	0	0	0
Cordycepin (purine analogue)	10	76	60	ND‡	ND‡
	100	30	4	58	24
Lampit (5-nitrofuran)	1	106	91	90	97
	10	6	0	0	0
	100	0	0	0	0
SQ 18506 (5-nitrofuran)	1	0	0	0	0
Metronidazole (5-nitroimidazole)	10	102	98	ND‡	ND‡
	100	96	47	40	46
Ro 7-1051 (2-nitroimidazole)	1	66	70	95	89
	10	48	9	56	4
	100	7	0	0	0

*Detailed chemical structures in ref. 1.
†Abnormal appearance.
‡Not determined.

T. dionisii (from J. R. Baker, Molteno Institute, University of Cambridge) and the Sonya strain of *T. cruzi*[5] were maintained in modified LIT medium[6] at 28 °C. This was supplemented with 10% (by volume) heat-inactivated new-born calf serum for *T. cruzi* and with the same concentration of heat-inactivated rabbit serum for *T. dionisii*. Four-day-old cultures of each organism were used to inoculate fresh medium containing drugs to an initial cell density of 10^6 organisms per ml. The effects of the drugs on growth were observed over a 72 h incubation period. The drugs used included representatives of each of the eight chemical structures so far tested clinically against *T. cruzi*[1]. The results (Table 1) showed that the drug sensitivities of the two species are almost identical. This suggests that *T. dionisii* can be used to replace *T. cruzi* in drug screens of this kind. Primary *in vitro* drug screens, however, are never entirely satisfactory since some false-positive and, more significantly, a few false-negative results will appear[7]. The absence of activity with Bayer 7602 Ac, which is quite active in animal screens and the detection of activity with cordycepin, which is relatively inactive *in vivo*, indicates that *T. dionisii in vitro* is not an exception to these limitations. But, if the alternative is no routine testing at all, as seems to be the situation now[1], we suggest that the use of *T. dionisii in vitro* as a safe, quick and inexpensive primary screen for detecting activity against Chagas' disease should be considered.

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Eosinophils and not lymphoid K cells kill *Trypanosoma cruzi* epimastigotes

CHAGAS' disease caused by the protozoan *Trypanosoma cruzi* remains a medical problem of immense proportions in Latin America, and yet the study of the pathogenesis and immunology of the disease can reasonably be said to be in its infancy. The demonstration of the protective role of humoral immunity *in vivo*[1] and of antibody-dependent cell-mediated cytotoxicity against *T. dionisii in vitro*[2] has raised the question that this phenomenon may be important biologically in Chagas' disease and might be mediated by K cells normally assayed as killer cells against antibody-coated tumour cells[3]. Here we address the second part of the question, and show that K cells in rat spleen have no detectable activity against *T. cruzi*, but that the activity resides in cell preparations rich in eosinophils.

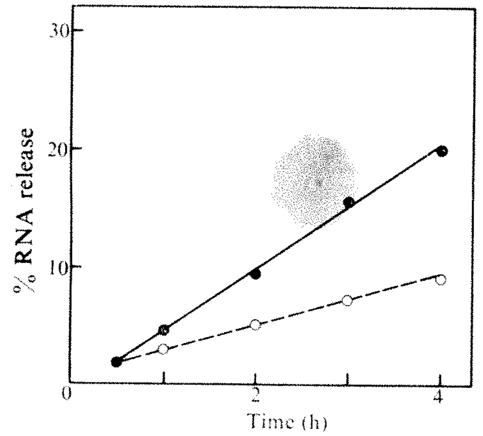

Fig. 1 Showing the time course of cytotoxicity of normal rat spleen cells in the presence (●) and absence (○) of antibody. An inbred line of white rats (O strain) was used. The antiserum was prepared by injecting rats with 10^6 blood forms from parasitaemic mice, and bleeding at 14 d. Culture forms of *T. cruzi* (Y strain) were inoculated into 1 ml of Warren's medium[9] containing 10 μCi of ^{3}H-uridine (43 Ci mmol^{-1}). After 5 d, the parasites were washed four times with medium (RPMI:1640 containing 10 mM HEPES and 5% inactivated foetal calf serum), resuspended to a concentration of 10^6 ml^{-1} and distributed (100 μl) in small plastic tubes. Spleen cells were added in a volume of 100 μl to give a ratio of 20:1 (cells:parasites). The antiserum diluted 1:100 was added as a further 100 μl (total volume 300 μl). The tubes were incubated at 37 °C, and at the times indicated, tubes were removed from the incubator, resuspended and centrifuged. After centrifugation, 200 μl of the supernatant was carefully removed to a counting vial. Total isotope in 10^5 parasites was determined by placing 100 μl of the original parasite suspension in a counting vial. The parasites were lysed by adding 100 μl of 1% Triton X-100. A 2-ml aliquot of a water-miscible scintillation fluid (1 vol. Triton X-100 and 2 vol. toluene-based scintillation fluid) was then added to each vial. The vials were counted in a liquid scintillation counter, and after correcting for background, % RNA release was calculated: $1.5C \times 100/T$ (T = the total counts in 10^5 parasites (mean of 4 replicates) and C = counts in each test vial (2/3 of supernatant counts)). The results were analysed by analysis of variance using logarithmic transformation, and Duncan's multiple range test[10]. All points shown are significantly different at the 5% level or less. Control release in the presence of antibody but absence of cells was not significantly different from (○), and has not been plotted.

It has been necessary to develop a simple and economical isotopic technique to assay parasite death. It has been noted previously (and we have confirmed it) that trypanosomes do not incorporate sufficient ^{51}Cr to provide a cytotoxic assay[2]. Those authors described a technique using ^{99m}Tc, but the limited availability and short half life (6 h) of this isotope provide considerable practical difficulties. Based on experiments with tumour cells where the release of RNA was found to provide a sensitive assay for cytotoxicity[4], we investigated the use of ^{3}H-uridine to label the RNA in the parasite. We have found (to be published) that, as is the case with tumour cells, RNA is released more rapidly than DNA and more completely than protein from killed parasites. Culture forms (largely epimastigotes) were labelled by growing them in a medium containing ^{3}H-uridine and >95% of the incorporated label was precipitable by 5% trichloracetic acid (and thus RNA). Figure 1 shows the release of RNA from *T. cruzi* by normal rat spleen cells in the presence and absence of specific antibody to the parasite, in medium containing inactivated foetal calf serum.

To investigate the properties of the cells mediating this cytotoxic reaction, the spleen cells were fractionated by Ficoll–Hypaque into high and low density fractions[5], followed by nylon wool columns to separate the cells on the basis of their adherence properties[6]. These fractions were tested against antibody-coated P815 cells as an assay for K cells, and against antibody-coated *T. cruzi*. It has already been shown that K cells are low density, non-adherent cells[4,7].

The activity against *T. cruzi* (Fig. 2) is found in the high density fraction (*P*) which contains very little activity against the

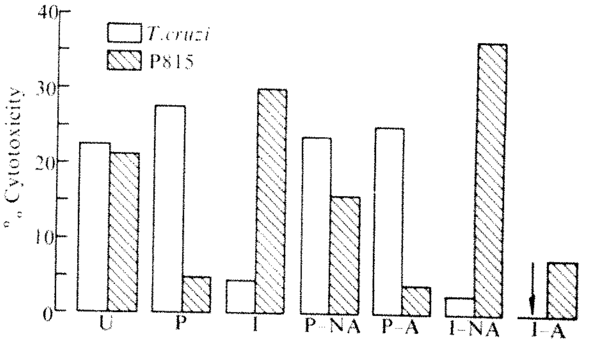

Fig. 2 Comparison of cell fractions against P815 mastocytoma cells using a ^{51}Cr release assay[4] and *T. cruzi* using the RNA release assay described in Fig. 1. Each fraction was tested in the presence and absence of the specific antibody for the appropriate target. U, Unfractionated rat spleen; P, pellet; I, interface after Ficoll–Hypaque separation[5]; P–NA, pellet cells after passage through nylon wool (non-adherent); P–A, pellet cells recovered from the nylon wool column (adherent cells); I–NA, interface cells non-adherent to nylon wool; I–A, interface cells adherent to and recovered from nylon wool. Nylon wool was used as described previously[6,7]. Assay against *T. cruzi* was carried out at a ratio of 20:1 for 4 h and against P815 was carried out at a ratio of 40:1 for 3 h after light centrifugation. % cytotoxicity was calculated from % isotope release: (test − control)/(maximum − control), where test = % release in the presence of antibody, control = % release in the absence of antibody, maximum = release after freezing and thawing (100% for RNA from *T. cruzi* and 81% for ^{51}Cr release from P815). The control values were 12.0% for *T. cruzi* and 15.5% from P815. None of the control values was more than 3% higher than the spontaneous release (tubes containing antibody but not effector cells). The arrow indicates a value where the difference between the presence and absence of antibody was not significant at the 5% level.

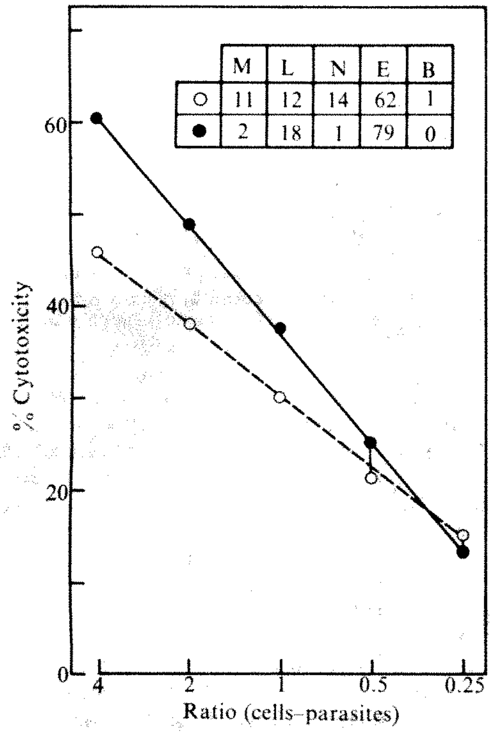

Fig. 3 An experiment using cells induced with dextran (molecular weight 70,000) and rich in eosinophils. Rats were injected with 5 ml of 6% dextran 15 h before collection of the exudate. The cells were tested unfractionated (○) and after carbonyl iron treatment (●) to remove adherent cells, by incubating 5 ml of cell suspension with 50 mg of carbonyl iron for 15 min at 37 °C. The non-adherent cells were recovered using a magnet. Differential cell counts were made after staining with May–Grunwald–Giemsa, and the values are the mean counts made by three different individuals. M, macrophages; L, lymphoid cells; N, neutrophils; E, eosinophils; B, mast cells. The two lines are significantly different at the 1% level, although the individual points joined by a vertical line are not significantly different at the 5% level. Linear regression analysis; (○) slope 5.96+0.36, correlation coefficient 0.986; (●) slope 8.99+0.29, correlation coefficient 0.996.

tumour cells. On the other hand, very little activity against *T. cruzi* is found in the low density fraction (*I*), which contains most of the activity against the tumour cells. The two activities are virtually mutually exclusive, as can be seen by comparing the activities in the pellet (P) with the interface cells (I) or the interface non-adherent cells (I–NA). This indicates that K cells have insignificant activity against *T. cruzi*, and that the cells active against the parasite have insignificant activity against these tumour cells. The high density of the active cells suggested that they may be granulocytes. To investigate this possibility, we induced peritoneal exudates with high proportions of granulocytes and tested them as effector cells against antibody-coated *T. cruzi*. Preliminary experiments with exudates rich in neutrophils or eosinophils suggested that eosinophils were the effector cells, and this can be seen in Fig. 3 where unfractionated exudate cells with 62% eosinophils shows spectacular activity against *T. cruzi* (a ratio of 1:1, giving 30% cytotoxicity in 4 h). Treatment of this exudate with carbonyl iron and a magnet to remove macrophages and neutrophils resulted in an increase in the activity against *T. cruzi* (a ratio of 1:1 giving 37.5% cytotoxicity). These types of experiments show that eosinophils can kill *T. cruzi*, but do not completely exclude other cell types from a subsidiary role.

In summary we have described an isotopic technique for assaying the killing of *T. cruzi* epimastigotes in which the criterion of parasite death is the release of macromolecular RNA. Apart from the obvious advantage that the assay measures the release of one of the parasites' natural macromolecules, and so can be taken as a good indicator of lysis, the fact that RNA is almost totally released when the parasites are lysed makes the assay sensitive. We have used the assay to investigate the nature of the effector cell killing *T. cruzi* in an antibody-dependent, complement-independent system. Eosinophils show strong activity, whereas lymphoid K cells seem to have insignificant activity.

Whether this eosinophil-mediated killing plays any biological part in the pathogenesis of American trypanosomiasis remains to be tested, but like experiments with *Schistosoma mansoni*[8], the present report adds another biological activity to our knowledge of the eosinophil.

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Note added in proof: We have been unable to induce eosinophil exudates with dextran in other strains of rats.

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Type C virus expression and host response in diet-cured NZB/W mice

THE immune complex renal disease of NZB/W mice is largely prevented and survival of the animals markedly prolonged by protein or calorie restriction[1,2]. These same dietary restrictions depress the antibody response[3-7] and inhibit the development of spontaneous cancer[8-10] in experimental animals. Spontaneous release of endogenous type C viruses (MuLV) and the autogenous host humoral immune response to these MuLV have been implicated in the aetiology of autoimmune disease in NZB and (NZB×NZW)F_1 (B/W) mice[11-14]. We wondered whether the disease-sparing effect of a low protein diet in B/W mice was attributable to a decrease in the production of endogenous MuLV or a reduction in the host response to this virus. Our findings suggest that neither of these events is responsible for the disease prevention.

Female B/W mice, raised in our laboratory breeding colony[15], were maintained from 4 weeks of age on a low phenylalanine-low tyrosine diet. This diet prolongs survival and prevents immune complex nephropathy in B/W mice[1]. Control mice were fed an unrestricted *ad lib* diet. Mice were necropsied when sick or at termination of the experiment and tissues were studied by light microscopy. The low phenylalanine-low tyrosine diet led to a significant prolongation of life (Fig. 1). The median survival on this restricted diet was more than doubled, that is, 24.3 months compared to 9.7 months for mice on the *ad lib* diet, and the relative death rate[16] was nearly fivefold less than in the *ad lib* diet group ($P < 0.001$). Of 23 mice in the restricted diet group, 15 were killed while still healthy at 2 yr

Table 1 Type C virus recovery from tissues cultivated from female B/W mice on amino acid restricted and *ad lib* diets

	Virus isolations			
Diet	Xenotropic	General titre (FFU ml^{-1})	Ecotropic	General titre (PFU ml^{-1})
Low amino acid	3/3	1,000	1/3	10,000
Ad lib	5/5	1,000	2/5	10,000

Values represent the number of mice which yielded virus/number tested. Assay for X-tropic virus in B/W mice was performed by initially co-cultivating mouse tissues with nonvirus producing murine sarcoma virus (MSV) transformed rat cells. Spleen, thymus and kidney tissues were co-cultivated from each mouse. After 7 d, the supernatant of these cultures was filtered and assayed for focus forming virus on rat or human cells[17,18]. Titre is expressed as focus forming units (FFU) per ml and is the general titre observed with cultures of the individual organs. The quantity of X-tropic virus produced by the mouse cells correlated with the amount of pseudotype MSV produced by co-cultivation[27]. Ecotropic virus produced by the mouse tissues was assayed by the standard XC plaque technique[28]. The titre is expressed as plaque forming units (PFU) per ml.

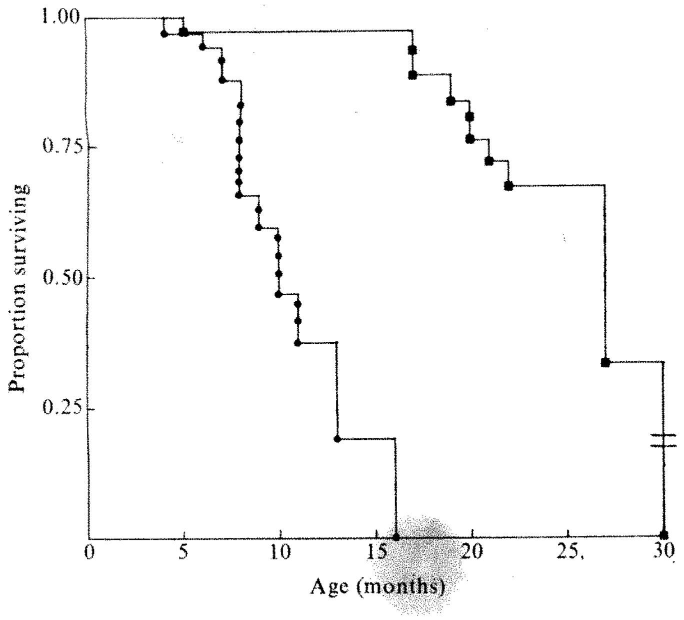

Fig. 1 Survival curves of B/W female mice on amino acid restricted (■) and *ad lib* (●) diets. The amino acid restricted group contained 35 mice and the *ad lib* diet group 32 mice. The data include killed and dead mice under long term observation as well as those sacrificed at 8 months of age. The survival curves and relative death rates were calculated using the actuarial life table approach and observed numbers of death in each group were compared to their expected numbers, assuming that no difference existed between groups[16].

of age and minimal glomerulonephritis was observed in only 3. Of the 8 mice in this diet group that were found dead between 5 and 30 months of age, 3 showed moderate glomerulonephritis and the other 5 died of unrelated causes. Thus, about 75% (17 of 23) of the diet-restricted mice were free of renal disease at an average age of 20 months. In the *ad lib* group, by contrast, all (29 of 29) mice had severe glomerulonephritis at an average age of 8 months. In general, the glomeruli in the diet-restricted group were neither enlarged nor hypercellular and there was no thickening of the mesangial matrix or capillary basement membranes. Immunoperoxidase staining also demonstrated a marked decrease of immunoglobulin deposition in the kidneys of the restricted diet mice compared to the control mice (data not shown). We thus confirmed that diet-restriction has a profound inhibitory effect on the development of immune complex (IC) nephritis in B/W female mice ($P < 0.001$). A similar preventive effect upon malignancy was not noted since lymphomas were found in two of the amino-acid restricted B/W mice at 21 and 24 months of age, whereas no lymphomas were found in the shorter lived B/W mice on the *ad lib* diet.

To determine whether the diet had any influence on production of endogenous type C virus we assayed tissues of amino acid-restricted B/W females aged 19–23 months and compared their virus expression with that of B/W controls on an *ad lib* diet. It has been shown that all cells from NZB and B/W mice produce in substantial titres an endogenous xenotropic (X-tropic) virus that can productively infect only cells from a heterologous species[13,14,17,18]. Tissues from the B/W hybrid sometimes also produce an endogenous ecotropic virus, present in the NZW parent, which infects and spreads readily in mouse cells[13]. As shown in Table 1, the prevalence and titre of X-tropic virus released by different tissues of the amino acid-restricted B/W mice were equivalent to those of control B/W mice. It is apparent that the dietary prevention of IC nephritis in B/W mice was not linked to any decrease in the production of endogenous X-tropic virus as measured by our *in vitro* technique. Moreover, since these diet-restricted mice had almost no signs of IC nephritis 1–11 months after the virus titre in their littermates was assessed, high production of X-tropic virus did not correlate with ultimate development of kidney lesions. In two controls and one experimental mouse, ecotropic AKR-MuLV was also detected. The presence of this virus was not associated with any specific pathological changes in the kidney.

We next determined whether the prevention of IC nephritis by the restricted diet correlated with any reduction in the host

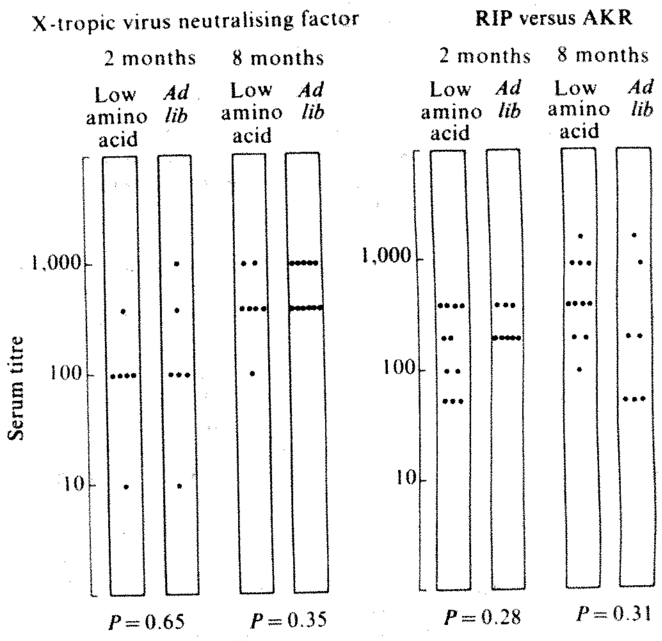

Fig. 2 Levels of anti-viral activity in female B/W mice fed amino acid restricted and *ad lib* diets. Sera were diluted 1:5 in phosphate buffered saline. Neutralisation activity was measured by reduction of foci on NRK cells of an NZB pseudotype sarcoma virus bearing the X-tropic virus type specific envelope antigen, as described[18,23]. The radioimmune precipitation assay of intact labelled ecotropic (AKR) virus has also been described[20]. At 8 months of age severe glomerulonephritis was present in all controls but was totally absent in those mice fed the restricted diet.

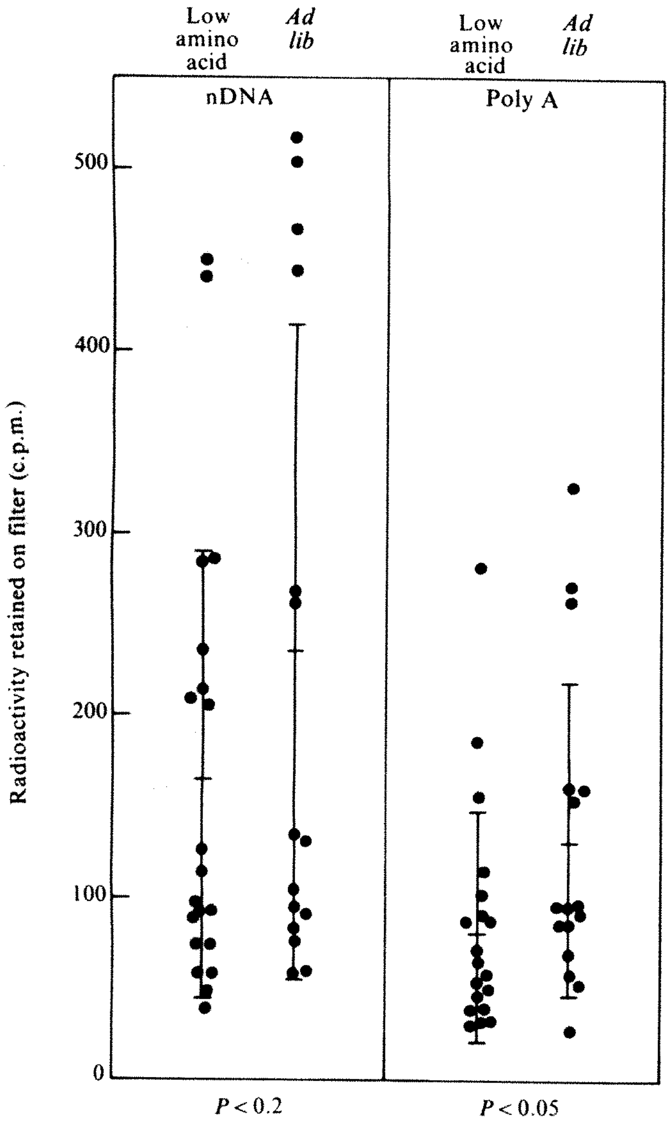

Fig. 3 Serum anti-nucleic acid antibodies in 8 month old female B/W white mice fed amino acid restricted and *ad lib* diets. Antibodies to native DNA (nDNA) and polyribonucleic acid (poly A), a single stranded RNA, were determined by a cellulose ester filter radioimmunoassay[29]. 100 μl of a 1:10 dilution of serum in minimum essential medium was heat inactivated at 56 °C. After adding 10 μl of ^{3}H-DNA (116 ng, 800 c.p.m.) or ^{3}H-poly A (7 ng, 800 c.p.m.), the reaction mixtures were incubated at 37 °C followed by incubation for 18 h at 0 °C. The reaction mixtures were diluted and passed through Millipore filters. The filters were washed, dried, and the radioactivity retained on the filters was counted by liquid scintillation.

response to endogenous viruses. A breakdown in the control of endogenous type C viruses could lead to high tissue and serum concentration of these viruses and the resultant hyperresponse to one or more of the viral antigens, for example, envelope glycoproteins (gp70), might contribute to the renal glomerular immune complex deposition[11,19]. Binding antibodies to intact labelled AKR ecotropic virus have been found in the sera of B/W mice[20] but no antibodies to X-tropic virus have been detected[21]. But, a serum non-immunoglobulin factor associated with mouse lipoproteins has been identified which neutralises specifically this X-tropic class of endogenous MuLV (refs 22, 23). No significant differences were found in the titre of either X-tropic virus neutralising factor or antibodies to the AKR-MuLV in sera from the mice in amino acid-restricted or *ad lib* diet groups (Fig. 2). In both diet groups the X-tropic virus neutralisation titres increased somewhat with age. A similar age increase in the AKR precipitating antibody titre was noted in the amino acid restricted but not in the *ad lib* group. In general, the geometric mean titres (g.m.t.) and the titre range of both X-tropic virus neutralising factor (g.m.t. 500, range 100–1,000) and anti-ecotropic virus antibody (g.m.t. 320, range 100–1,600) were similar in both diet groups at 8 months of age to those values previously detected in untreated NZB and B/W mice[14,20,24]. Although subtle differences might yet be revealed if these analyses were done on a larger number of mice, these findings do suggest that the diet-induced prevention of immune complex nephritis in B/W mice is not due to any major decrease in production of X-tropic virus neutralising factor or precipitating antibodies to the endogenous ecotropic virus.

Another characteristic of the autoimmune disease complex in B/W mice is antibodies to nucleic acids. We found the mean binding of serum antibodies to native DNA and poly A was somewhat lower at 8 months of age in the group fed the amino acid-restricted diet compared to the control group (Fig. 3); this reached statistical significance ($P < 0.05$) only to poly A.

These data suggest that the dietary-induced increase in longevity and prevention of immune complex disease in female B/W mice is unrelated to any influence upon production of endogenous type C viruses or the autogenous host response to these viruses. A similar lack of association between autoimmune markers and high titres of xenotropic virus was found in F_1 progeny of crosses between NZB mice and other strains of laboratory mice[25]. If one assumes that virus production *in vivo* is accurately represented by the *in vitro* virus assays, these observations suggest that the IC renal disease in B/W mice is not solely a consequence of spontaneous oncornavirus production. The trend towards reduction in antibodies to nucleic acids observed in the diet-treated mice, although not profound, may have contributed to the reduction in IC nephritis since about half of the immunoglobulin deposited in glomeruli of control B/W mice reacts with nucleoprotein[26]. Measurement of the total immunoglobulin levels, the types of immunoglobulins produced, and the quantitation of circulating immune complexes might clarify further whether the diet-induced disease-protection involves a generalised decrease in antibody synthesis. The diet may decrease certain species of immunoglobulins or complexes other than those containing viral or nuclear antigens. These studies suggest, however, that since the diet did not significantly affect the host response to endogenous viruses or nucleic acids, other factors such as hormone levels and the number and function of cells responsible for mediating the immunological reactions may have been affected.

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Epizootiology, transmission and approach to prevention of fatal simian haemorrhagic fever in rhesus monkeys

RHESUS monkeys (*Macaca mulatta*) and all other macaques are extremely susceptible to the fatal disease, simian haemorrhagic fever (SHF). Introduction of this agent into a laboratory colony will result rapidly in the complete loss of all macaques in that colony[1-6]. An outbreak in one of our rhesus colonies resulted in the loss of all 212 animals in a period of 12 d. Because of this great loss and its consequences, we initiated an investigation into the epizootiology, transmission and prevention of this disease. These studies have shown that the virus is carried as a chronic infection in patas monkeys (*Erythrocebus patas*) with no apparent signs of disease. It can be transmitted to the rhesus monkeys by accidental inoculation with tissue fluids, and once they are infected, spread by aerosol and contact is rapid.

Clinically, SHF is a febrile, haemorrhagic disease of macaques caused by an RNA virus which has been reported to be a togavirus[5]. The disease is characterised by rapid onset, early fever, mild facial oedema, failure to eat or drink, dehydration, protein in the urine, cyanosis, haemorrhages of the skin, bloody diarrhoea, nosebleeds, occasional bleeding in the orbit of the eye and subsequent death in most cases. Pathological changes associated with the disease include widespread small to large skin haemorrhages, haemorrhagic tissue death of the first few centimetres of the small intestine, enlarged spleen, intravascular coagulation and generalised destruction of lymphoid tissue[3,7].

The epizootiology of SHF has been described for eight outbreaks[1,2,6]. The disease has only been observed in macaques. The first seven outbreaks involved recently imported rhesus monkeys with subsequent spread of the disease to other macaque species. The source of the eighth outbreak was not identified. All of the epizootics were characterised by an explosive onset, rapid spread within exposed groups and a high case–fatality ratio. No human illness was associated with either the current outbreak or previously reported epizootics of SHF.

In November and December 1972, an outbreak of SHF occurred in our colony in a building housing two groups of monkeys: 212 rhesus monkeys and 46 patas monkeys. The two groups were kept in separate rooms connected by an open loft : no other animals were housed in the building. Only the rhesus monkeys were involved and of the 212 animals, 172 had been in the colony for 6 months to 1 yr. The other 40 rhesus monkeys were introduced into the colony in a one-week period. The new animals had been conditioned for 60 d at the facilities of an animal importer, during which they were held in two separate rooms with no other animals. The patas monkeys had been in our colony for 2 yr.

Except for diarrhoeal disease in 10 of the newly arrived rhesus monkeys, no major disease problems were noted in the colony until about 30 d after the last animal was introduced. Suddenly, within one day, 60 rhesus monkeys, including all 40 newly arrived animals, developed a clinical illness characterised by depression and loss of appetite. The following day, an increasing number developed a similar illness. Many of the animals observed to be ill the previous day had developed facial cyanosis and showed dilated pupils; 15 of the animals were too weak to stand. The first fatalities occurred 3 d after the onset of illness, when 10 animals were found dead. Within 4 d, all remaining rhesus monkeys were affected clinically, and nine additional monkeys had died. In 12 d, a total of 106 monkeys had died. At this time, all surviving macaques were in advanced stages of illness and so were killed. At necropsy the affected animals had lesions characteristic of SHF. At no time did the patas monkeys exhibit signs of disease.

The disease seemed to have spread within the rhesus colony by direct and indirect contact exposure. Although all monkeys in the colony were in individual cages separated by a solid metal partition, they could touch each other by reaching around the partition. Also, all the female rhesus monkeys were weighed twice weekly as part of a nutritional experiment. For this, the animals were placed in one of three portable cages for transport to the scale. The portable cages were washed and autoclaved at the end of each day. Indirect contact of the monkeys did, however, result during use of these transfer cages for several animals on a given day. On some occasions, the same cages were also used to transport the patas monkeys to the scale.

Although spread by means of needles used in the treatment and tattooing of monkeys had been incriminated in previous epizootics of SHF, exposure by this route in this outbreak was investigated carefully and found to be limited to a small number of animals. The 40 newly arrived rhesus monkeys were tattooed using the same equipment on or shortly after arrival. The tattoo needle was cleaned with alcohol between animals. A few animals in the newly arrived group may have been given injections of vitamins and antibiotics with a common needle. Most of the infected animals, however, had not been tattooed or inoculated for a number of months before the outbreak.

In this outbreak, approximately 90 d (60 d at importer's and 30 d in our facility) had elapsed between importation of the newly introduced group of monkeys and the onset of the epizootic. Disease compatible with SHF had not been noted since 1965 at the animal importer's facilities and the importer had not heard of any similar disease problem in monkeys sold to other customers. In experimental studies the incubation period of SHF has ranged from 3–7 d after inoculation, with most deaths occurring within 7–13 d (refs 2 and 7). The short incubation period of the disease, the long time which elapsed between importation of the newly introduced monkeys and the onset of the epizootic, and absence of the disease in animals at the importer's facilities suggest that the rhesus monkeys were first exposed to the disease at the NIH colony. Studies on the transmission of the SHF were initiated and demonstrated that the disease could be spread from infected to uninfected rhesus monkeys by direct contact with animals in the same cage or by aerosol to animals in adjacent cages, as well as by inoculation. With inoculation, the animals died in 7–12 d.

In view of the extreme susceptibility and lethality of SHF in rhesus monkeys it is likely that this is not a natural epizootic disease of macaques. Once introduced into macaques either in the wild or laboratory colony, the disease would destroy essentially all animals in two weeks. The few remaining animals seen in laboratory conditions following a long period of supportive treatment are not chronic carriers of the virus, but have high levels of antibodies[4].

The investigation of the possible source and method of introduction of the infection into our rhesus population was fortunately made easier because only one other species of sub-human primate was in the building. For this reason, careful study of the patas monkeys was also initiated to determine if they were the source of the infection. Tests showed that when serum from some patas was inoculated intravenously into a rhesus monkey, SHF and death resulted in 4 d. In a subsequent series of studies, it was found that approximately 10% of recently imported patas monkeys were chronic carriers of SHF. Minute quantities of blood or tissue fluids could transmit the infection to the rhesus. Transmission did not occur between patas and rhesus when the animals were housed together in the same cages, so it obviously required direct mechanical transmission of infected tissues and did not occur by contact or aerosol. It seems that our epizootic was initiated by the use of a multidose vial which was used to inoculate animals of both species. The inappropriate re-introduction of a contaminated needle from animals with more than 10^{-12} ID (infective doses) per ml of blood into the vial following the injection of a chronically infected patas would have been sufficient to contaminate the vial contents and spread the disease to rhesus monkeys which were subsequently infected with material from the same vial.

Prevention of SHF can be accomplished by interrupting transmission from the natural reservoirs of infection. Transmission of SHF on this occasion occurred because asymptomatic patas monkeys, which chronically carry this virus, were maintained in the same building by the same personnel as rhesus monkeys and, unfortunately, because of a technical error, some of the contaminated tissue fluids from such a patas monkey was inoculated into a rhesus. Once the rhesus animal was infected, the disease spread through the rhesus colony by direct contact and aerosol. Thus, prevention of SHF in such colonies must include meticulous attention to prevent procedures which might transmit infected tissues from patas to rhesus. Whenever possible, the two species should be housed in separate facilities to minimise further the risk of cross inoculation.

Patas animals can carry this deadly virus for at least 2 yr, and we have also found that approximately 1% of baboons (*Papio papio*) and African green monkeys (*Cercopithecus aethiops*) we have studied carried latent SHF. To date, there has been no evidence that chimpanzees carry this virus. All other African species, however, should be considered possible carriers. Importations and shipments of monkeys must also be arranged to separate the African species from macaques since inadvertent exposure to blood or tissue fluid might occur in open wounds during shipment. Since SHF is a togavirus, the possibility of arthropod-borne transmission must also be investigated.

We have also reported that the complex of polyriboinosinic–polyribocytidylic acid-containing poly-L-lysine and carboxymethylcellulose is effective in preventing the spread of SHF in colonies of macaques if used in recently exposed animals[8]. Animals showing signs of disease however, were not helped by using this drug.

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Y-forms as possible intermediates in the replication of measles virus nucleocapsids

THE genome of measles virus, a paramyxovirus, is a non-segmented single stranded RNA molecule which is enclosed within a helix of protein subunits to form a linear nucleocapsid[1]. Little is known about its mode of replication. It is known, however, for other paramyxoviruses that during the growth cycle transcription of the RNA occurs from an intact nucleocapsid by a virion-associated enzyme[2–4]. The resulting messenger RNA molecules are less than the full genome length and are unencapsidated[5]. By contrast, during viral RNA replication no free genome length RNA can be detected[5,6] and both plus and minus-stranded RNA are found only in the encapsidated form[7]. From this evidence, Kingsbury has suggested[7] that nucleocapsids of paramyxoviruses replicate without separation of the protein and nucleic acid portions of the template nucleocapsid and has implied that plus and minus-stranded RNAs enclosed within the capsid structure act as templates for each other. If this is so, association of the two types of nucleocapsid in a replicative form should be detectable in infected cells. We have conducted a search by electron microscopy for such structures in measles virus infected cells and the results are described here.

Vero cell monolayer cultures were infected with the P9 strain of measles virus[8] at a multiplicity of 1 pfu cell^{-1}. Preparations of nucleocapsid material for electron microscopy were obtained by lysis of the monolayers with a few drops of 2% phosphotungstic acid at 18–24 h post-infection, the time of maximum nucleocapsid synthesis. A small drop of the lysate was applied to a carbon-Formvar coated grid, blotted dry with filter paper and viewed in the electron microscope. Nucleocapsid lengths were measured on tracings of enlarged electron micrograph negatives or by counting capsid helix turns.

The majority of nucleocapsids observed were linear molecules. However, rare Y-shaped structures with two arms of equal and variable length were also observed (Fig. 1). Such structures would be expected for a replication mode in which one strand was being copied from one end. The variable lengths of the arms indicate that the structures represent different stages of replication.

From a large number of experiments, 26 structures were selected which we considered to be unambiguous Y-forms. In those with very short arms (Fig. 1*a*, *b*), the number of turns of the capsid helix was the same, ±1, for both arms. In those with longer arms, the lengths differed only by an amount probably consistent with the deformations introduced during preparation. The total length of the 'unreplicated' stem portion

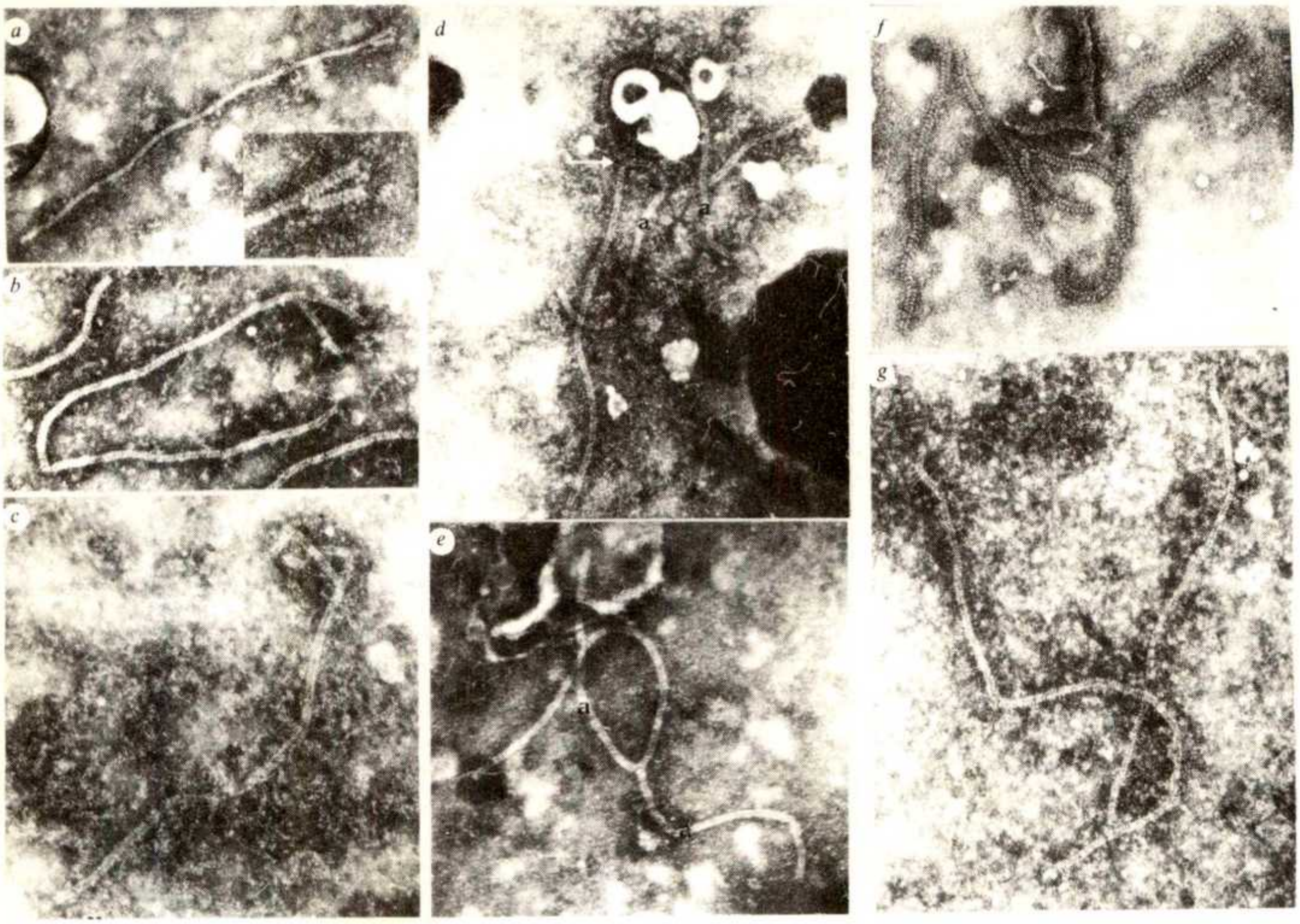

Fig. 1 Y-form nucleocapsid at various stages of replication. Lengths of arms and stems, respectively. *a* ×39,600 (inset ×99,000). 0.07 μm, 0.07 μm and 1.45 μm. Nine turns of capsid helix on each arm. *b* ×66,000. 0.12 μm (21 turns), 0.13 μm (22 turns) and 1.34 μm. *c* ×92,400. 0.18 μm, 0.16 μm and 0.7 μm. *d* ×66,000. 0.73 μm, 0.73 μm and 0.6 μm. Arrow indicates junction of arms (**a**) and stem. *e* ×66,000. 0.5 μm, 0.53 μm and 0.8 μm. *f* ×66,000. 0.34 μm, 0.33 μm and 1.21 μm. *g* ×66,000. 1.26 μm, 1.07 μm and 0.13 μm.

plus one arm was between 0.8 and 1.6 μm. This is similar to the range of lengths found for non-replicating nucleocapsids in the same preparations. The variation in length of the 'unreplicated' portion of the Y-forms was apparently random. Molecules adventitiously joined in suspension or on drying on the grid can generally be distinguished by the overlapped appearance of the junction and by the lack of relationship between the lengths of the three arms.

A feature apparent in most (22 out of 26) of the 'replicating forms' is that the capsomeres in one arm of the Y-forms have the opposite orientation to those in the other (Fig. 1*a*, *b*, *f*, *g*). This might be expected from the presumed opposing polarities of the encapsidated complementary RNA chains. If, however, one of the arms had turned over, the orientation of capsomeres would be the same in both arms (Fig. 1*d*, *e*). Assuming that the RNA is replicated by presently known processes, the new chain beginning with the 5′ end would be copied from the 3′ end of the parent molecule. By reference to Fig. 1*b*, it can be seen that this allows identification of the 3′ end of the nucleocapsid as the end with the convex orientation of capsomeres.

The structural features at the junction are not yet apparent although some alteration of capsid arrangement is suggested by Fig. 1*a*, *b* and *g*. Better preserved preparations and higher resolution are required for further study.

Rigid proof that the Y-forms represent the true replicative form may be difficult to obtain since they occur only at very low frequency, presumably owing to the rapid rate of replication. Our attempts to increase the number of Y-forms by arrest of nucleocapsid synthesis by rapid freezing and by the use of inhibitors have so far been unsuccessful. These preliminary results, however, support the idea that the nucleocapsid is replicated from an intact nucleocapsid template with local unwinding to expose the enclosed RNA and rapid assembly of progeny nucleic acid and protein[7].

We previously reported the presence of circular nucleocapsid molecules in measles-infected cells[9]. Similar circular molecules, which were thought to be end-to-end aggregates of linear nucleocapsid, had been already reported for another paramyxovirus, NDV, by Compans and Choppin[10] but we were unaware of their work. It seems likely from the results presented here that circular nucleocapsids do not function in the replication of the normal linear nucleocapsid population. We have, however, found rare circular molecules which appear to have attached tails in even lower proportion than the Y-forms. These may represent templates functioning in a rolling-circle type of mechanism as previously suggested[9] but this requires further study.

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Presence of 1,25-dihydroxyvitamin D_3-glycoside in the calcinogenic plant *Cestrum diurnum*

A DISEASE clinically and morphologically similar to vitamin D-intoxication, characterised by hypercalcaemia and extensive soft tissue calcification, has been described in grazing animals and linked to the ingestion of leaves from several plant species including *Cestrum diurnum* (*C.d.*)[1-3] and *Solanum malacoxylon*[3]. Early reports indicated that activity resembling the hormonal form of vitamin D_3, 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$), existed in extracts of both of these plants[3-5]. The active calcitropic principle from *S. malacoxylon* has been identified as a 1,25-$(OH)_2D_3$-glycoside[6-8]. We report here the isolation and identification of a 1,25-$(OH)_2D_3$-glycoside in *C.d.* It is proposed that this principle accounts for the hyperabsorption of calcium found in animals eating this plant.

We have previously reported that the ingestion of *C.d.* results in increased calcium absorption and induced calcium binding protein (CaBP) synthesis in chicks fed a diet containing strontium[5]. Strontium blocks the renal 1-hydroxylase enzyme which converts vitamin D_3 to active 1-hydroxylated metabolites[9]. Hence, any plasma 1-hydroxylated-D-vitamins in these animals must originate exogenously. With the development of a specific radioreceptor assay for 1,25-$(OH)_2D_3$ (refs 10–12), it is now possible to directly assess the 1,25-$(OH)_2D_3$ activity in the plasma of chicks after *C.d.* administration. Table 1 reveals that after a single oral dose of *C.d.*, there is a rapid increase in plasma 1,25-$(OH)_2D_3$-like radioreceptor activity followed by an elevation in duodenal CaBP. Within 12 h the 1,25-$(OH)_2D_3$-like activity increases about eightfold over the normal physiological concentration of the hormone in chicks (5.2 ng dl^{-1}) (ref. 14). These observations suggest that the biologically active calcaemic principle of *C.d.* might be 1-hydroxylated in the native plant, since the 1-hydroxyl moiety of vitamin D analogues has been shown to be essential for efficient binding to the chick intestinal receptor protein used in the radioreceptor assay. Consequently, when this calcaemic plant factor is ingested by animals, it might circumvent the stringent renal regulation of the circulating level of the 1,25-$(OH)_2D_3$ hormone.

Celite liquid–liquid partition chromatography[12] was then used to analyse plasma from chicks treated with *C.d.* Aliquots of plasma obtained from the experiment described in Table 1 were combined with tracer radioactive 1,25-$(OH)_2D_3$ and chromatographed on Celite (Fig. 1*a*). The coincident migration of authentic hormone with the radioreceptor activity from the chick plasma further substantiates that *C.d.* must contain a factor very similar, if not identical, to 1,25-$(OH)_2D_3$.

Table 1 Appearance of plasma 1,25-$(OH)_2D_3$-like activity and intestinal calcium binding protein (CaBP) in chicks given an oral dose of *C. diurnum*

Time after dose (h)	Plasma radioreceptor activity (ng equivalent 1,25-$(OH)_2D_3$ per 100 ml)	Intestinal CaBP (μg per mg protein)
0	<0.5	0
6	24	<0.3
12	43	1.4
24	18	6.9

White leghorn cockerels were placed on a diet[5] supplemented with 1.2 IU vitamin D_3 per g, 2.56% Sr, 0.05% Ca and 0.76% P. Each chick received an oral dose of *C.d.* extract equivalent to 2 g original dried leaf. Plasma from five chicks was pooled, purified, and assayed for 1,25-$(OH)_2D_3$-like receptor activity as previously described[12]; data represent the mean of triplicate assays for each pooled sample. Duodenal CaBP was quantitated by radial-immunoassay[13] in supernatant fractions of homogenised duodenal mucosa, and the mean data expressed as μg CaBP per mg total supernatant protein.

Contrary to this idea that the plant factor is identical to 1,25-$(OH)_2D_3$ is the slightly slower observed appearance of intestinal CaBP after administration of *C.d.* as compared with that observed following administration of the pure hormone[15]. Also, we could not demonstrate the receptor activity for the crude *C.d.* extract that was predicted by bioassay of this material[5]. These observations suggested that some preliminary modification of the *C.d.* principle might be required in the animal before receptor binding and consequent biological activity. We have previously shown that hydrolysis of the

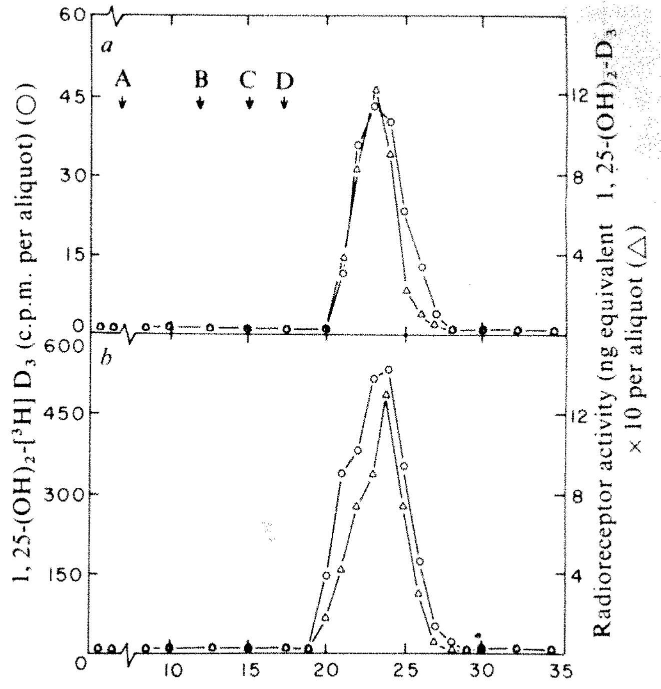

Fig. 1 Comigration on Celite of the lipid soluble 1,25-$(OH)_2D_3$-like factor from *C.d.* with authentic tritiated hormone. *a*, After an oral dose of *C.d.*, chick plasma was extracted with $CHCl_3$:CH_3OH (1:2) and partially purified via Sephadex LH-20, silicic acid, and microCelite chromatography[12]. The 1,25-$(OH)_2D_3$-like material from *C.d.* co-eluted with standard, tritiated 1,25-$(OH)_2D_3$ (5500 c.p.m., 0.32 ng) in each of these column systems (data not shown). The material was then subjected to a long Celite column (1×40 cm) (*a*) and the radioreceptor activity was compared to the migration position of the 1,25-$(OH)_2[^3H]D_3$ internal marker. Other vitamin D forms migrate as indicated: A, 25-hydroxyvitamin D_3; B, 24,25-dihydroxyvitamin D_3; C, 25,26-dihydroxyvitamin D_3; D, 1,25-dihydroxyvitamin D_2 (ref. 12). *b*, Dried *C.d.* leaf (300 g) was extracted with $CHCl_3$ (discarded) and the nonsoluble material was further extracted with $CHCl_3$:CH_3OH (1:2). The active material was precipitated with acetone and the pellet subsequently redissolved in $CHCl_3$:CH_3OH (1:2). After removal of this solvent, the material was suspended in citrate–phosphate buffer (*p*H 5.0) and 500 mg of mixed glycosidases derived from the liver of the sea worm, *Charonia lampas* (Miles), was added. The mixture was incubated for 16 h at 37 °C under N_2 and the material was placed into a separatory funnel with H_2O:CH_3OH:$CHCl_3$ (1:1:1.5) and the phases allowed to separate. The lower $CHCl_3$ phase was then combined with three $CHCl_3$ washes of the upper phase and dried in a rotary evaporator under N_2 in a vacuum. Bioassay identification of the active fraction was carried out by administration to strontium-fed chicks and measuring intestinal calcium absorption and calcium binding protein[5]. Tracer 1,25-$(OH)_2[^3H]D_3$ (15,000 c.p.m., 0.875 ng) was added to the dried, hydrolysed material and the mixture was pre-purified by silicic acid chromatography (1×20 cm) eluted with ether: acetone (95:5 v/v) under N_2 pressure. The active radioreceptor fractions were combined and purified by Sephadex LH-20, microCelite chromatography and, finally (*b*) a 1×40 cm Celite liquid-liquid partition column[12].

calcaemic factor from *S. malacoxylon*, by a mixture of glycosidases, has been a prerequisite for receptor binding[6]. Thus we exposed (*in vitro*) an extract of *C.d.* to this same mixture of glycosidases from the liver of the seaworm, *Charonia lampas*, and obtained a lipid-soluble biologically active fraction. This hydrolysed material was then purified by a series of chromatographic columns (see legend of Fig. 1). The final step was Celite chromatography and the 1,25-$(OH)_2D_3$-like binding activity comigrated with authentic tritiated 1,25-$(OH)_2D_3$ (Fig. 1*b*), as also observed with the purified plasma principle from *C.d.*-fed strontium-inhibited chicks (Fig. 1*a*). It is thus reasonable to assume that the *in vitro* hydrolysis by the mixed glycosidase preparation resembles a similar hydrolysis which takes place *in vivo*, both liberating an active fragment indistinguishable from 1,25-$(OH)_2D_3$ by all of the chromatographic systems used (see Fig. 1 legend).

The chromatography scheme used analytically in these comigration studies was then used to purify sufficient quantities of *C. lampas*-hydrolysed material to allow for further characterisation of the *C.d.* factor. The pooled peak fractions from the final Celite column exhibited an ultraviolet absorption spectrum identical to that of 1,25-$(OH)_2D_3$ (ref. 16). Quantitation of the material was achieved with the radioreceptor assay which indicated that 1.2 µg of 1,25-$(OH)_2D_3$-equivalents were present. This represents the total activity derived from 300 g of dried *C.d.* leaf, but this should not be used as an indication of the concentration of the factor in *C.d.*, since we were unable to assess yields during the early extraction procedures.

This evidence for the 1,25-$(OH)_2D_3$-like nature of the *C.d.* principle led us to compare the mass spectrum of the hydrolysed purified *C.d.* extract to that of crystalline 1,25-$(OH)_2D_3$ by gas chromatography/mass spectrometry (GC/MS). D-vitamins are known to pyrolyse during gas chromatography yielding two isomers—the 'pyro-' and 'isopyro-' forms, as a result of closure of the B-ring[8,17]; this fact is germane to understanding the fragment pattern. Figure 2*a* (inset) depicts the total ion current obtained with 1.2 µg of synthetic 1,25-$(OH)_2D_3$. The major peak of ion current emerges from the column at 7 min (arrow) and represents 1,25-dihydroxy*pyro*vitamin D_3; the second peak represents 1,25-dihydroxy*isopyro*vitamin D_3. The mass spectrum (Fig. 2*a*) of the major peak (arrow) shows characteristic fragments at *m/e* 380, 362, 251, 209, 155, 141, 105, 69, 59, and 55. A similar mass fragment pattern was observed for the second, '*isopyro*', peak of total ion current (data not shown). Figure 3 shows 1,25-dihydroxy*pyro*vitamin D_3 and the cleavages of the ABCD ring system which are typical of classic steroids[18] and probably produce the mass fragments actually observed (Fig. 2*a*) for 1,25-$(OH)_2D_3$.

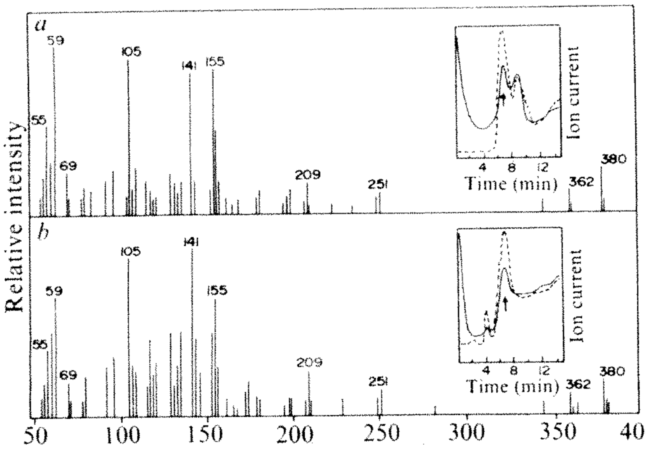

Fig. 2 Analysis of 1,25-$(OH)_2D_3$ and the lipophilic fragment of *C.d.* by gas-liquid partition chromatography and mass spectrometry. *a*, Gas chromatogram/mass spectrum of 1.2 µg of synthetic 1,25-$(OH)_2D_3$. *b*, Gas chromatogram/mass spectrum of 1.2 µg of purified hydrolysed *C.d.* Insets show gas chromatographic traces of total ion current (solid line) and ion current at *m/e* 380 (broken line). The arrows indicate the peak fractions which were characterised by mass spectrometry. These analyses were performed on a Model 3300-6110 Finnigan GC/MS with quadrapole analyser in the electron impact ionisation mode, equipped with a 2 mm × 50 cm, 3% OV-7 (100-120 mesh) glass column. Other parameters: injection port temperature, 260 °C; column temperature, 245 °C initial, 8 °C per min programmed increase; sample application in 5 µl double distilled ethanol (100%).

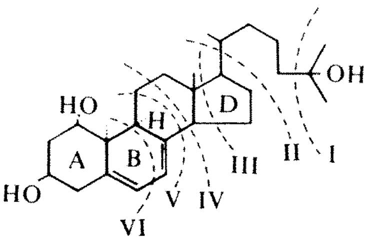

Fig. 3 Proposed fragmentation pattern for 1,25-dihydroxy*pyro*vitamin D_3. The molecular weight of 1,25-$(OH)_2D_3$ is 416, but the parent peak of the GC/MS, which is at *m/e* 380, represents the native molecule minus two hydroxyl groups that are lost in the form of H_2O during heating. Loss of the third hydroxyl in the form of H_2O yields the fragment at *m/e* 362. Cleavage I gives a major peak at *m/e* 59 by removal of the portion of the side chain; and, removal of the entire sidechain (111 AMU) from the fragment at *m/e* 362 (cleavage II) yields the peak at *m/e* 251 which is the fused ABCD ring array. As discussed by Budzikiewitz and coworkers[19], steriod ring systems are commonly cleaved between C_{14-15} and C_{13-17} (cleavage III) losing 42 AMU and leaving a fragment at *m/e* 209 in this instance. The *m/e* 209 fragment (or *m/e* 210 resulting from common hydrogen rearrangement in steroids[19]), now serves as a source for three complementary pairs of fragments which comprise the important remaining peaks of the 1,25-$(OH)_2D_3$ mass spectral pattern. These pairs include: *m/e* 155 and 55 resulting from cleavage at IV; *m/e* 141 and 69, from cleavage at V; and *m/e* 105 which represents both fragments generated from cleavage at VI. It should be emphasised that cleavages III-VI are characteristic cleavage positions predicted from the mass spectra of other steroids[18,19].

The inset of Fig. 2*b* illustrates the total ion current trace and the monitored ion current at *m/e* 380 obtained from gas chromatography of 1.2 µg of purified hydrolysed *C.d.* factor. Again, the major peak (arrow) emerges at 7 min, resolved from sample contaminants which apparently obscure any observation of the '*isopyro*-' form of *C.d.* Mass spectral analysis of this peak (Fig. 2*b*) reveals a fragment pattern equivalent to that obtained for 1,25-$(OH)_2D_3$. The coincident migration of the active lipophilic fragment of *C.d.* with 1,25-$(OH)_2D_3$ on the gas chromatographic column, and the identical mass spectra of the resulting peaks convincingly demonstrates that 1,25-$(OH)_2D_3$ is present in *C.d.*, although the steroid apparently exists in the native plant in the form of a glycoside.

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Synthesis, storage and release of acetylcholine by a noradrenergic pheochromocytoma cell line

DEVELOPING neural crest tissues show a certain degree of plasticity with respect to neurotransmitter synthesis. For example, heterotopic transplants of presumptive noradrenergic tissue may develop cholinergic properties and vice versa[1,2]. Also, monolayer cultures of rat sympathetic neurones which synthesise catecholamines may be influenced to synthesise acetylcholine by exposure to ganglionic non-neuronal cells or to medium conditioned by such cells[3,4]. Recently, a clonal cell line (designated PC12) was established[5] from a transplantable rat adrenal medullary pheochromocytoma[6]. This line expresses many characteristic properties of neural-crest-derived noradernergic adrenal chromaffin cells and sympathetic neurones, including synthesis, storage, uptake and specific release of catecholamines[5,7,8]. In addition, within several days after their exposure to nerve growth factor (or NGF, a protein which influences the growth and development of normal sympathetic neurones[9]), PC12 cells cease mitotic activity and extend long, neuronal processes[5]. We show here that noradrenergic cultures of PC12 cells can also synthesise, store and release acetylcholine.

PC12 cultures, maintained as described before[5,7] were assayed for choline acetyltransferase (CAT) activity by the method of Fonnum[10]. Assays performed in the absence of choline or in the presence of 100 μM naphthylvinyl pyridine (a specific inhibitor of CAT), gave values similar to tissue-free blanks, thus indicating the specificity of the activity for CAT and excluding the presence of carnitine acetyltransferase[11]. As shown in Fig. 1*a*, *b*, the specific activity (s.a.) of CAT varied markedly with cell density. At maximal cell density, specific activities reached 500–600 pmol per min per mg protein. This level is comparable with magnitude to that observed in rat brain tissue[12] but is lower than levels reported in chicken ciliary ganglia[13] (~50 nmol min^{-1} mg^{-1}). The increase in the s.a. of CAT in PC12 cultures occurred mainly during the logarithmic stage of cell growth and then plateaued when the cultures reached the stationary phase (Fig. 1*a*, *b*). Thus, the s.a. of CAT is apparently related to cell density rather than to the rate of cell division. As further evidence of this, CAT activities in cultures of division-arrested PC12 cells (produced by treatment with 1 μM cytosine arabinoside) were comparable to those in logarithmic growing cultures of similar density (see zero time in Fig. 1*c*, for example). Such findings suggest that the increase in specific CAT activity with increased cell density may be due to autoconditioning in a manner analogous to that observed for

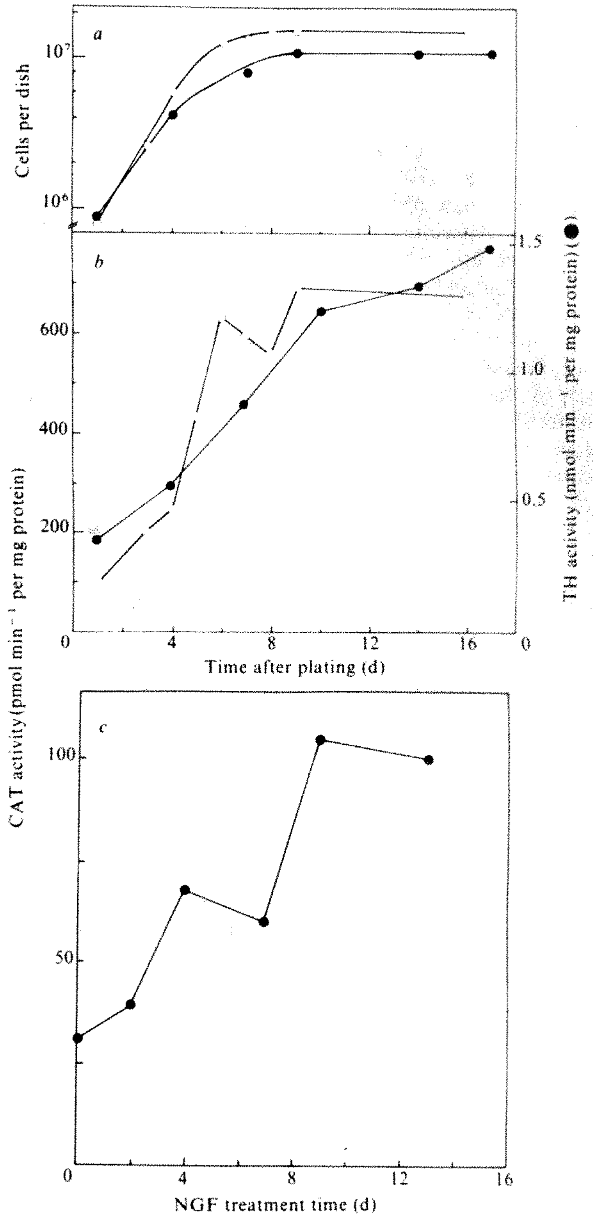

Fig. 1 Regulation of CAT activity in PC12 cells. *a*, *b*, Variation of specific activities of CAT (○) and TH (●) as a function of cell density. *a*, Cell number; *b*, specific enzyme activities. Cells were plated in collagen-coated 35-mm dishes as previously described[5]. In this, and in all subsequent experiments, the culture medium was changed every other day. At indicated times, cells were collected from duplicate sister cultures and assayed in duplicate for cell number and enzyme activity. CAT activity was measured according to Fonnum[10]; TH was measured according to Black *et al.*[19], with concentrations of tyrosine and DMPH_4 of 200 μM and 1 mM, respectively. Specific activities are based on protein levels of homogenates estimated (with a bovine serum albumin standard) by the methods of Lowry *et al*[20]. (TH assays) or McGuire *et al.*[21] (CAT assays). Variation in specific activities between duplicate cultures in this and other experiments was less than 15%. *c*, Effect of NGF treatment on the specific activity of CAT in division-arrested PC12 cells. Non-dividing PC12 cells were produced as follows. Stationary phase dishes (100 mm) of PC12 cells were exposed to 1 μM cytosine arabinoside (ara-C) for one week. The cells which survived this treatment (about 20% of the original population) were found to be non-dividing and to survive in the presence of ara-C for at least 1 month. The ara-C-selected cells were then subcultured into 35 mm collagen-coated dishes at low cell density (1.2×10^5 per dish) in the presence of ara-C. At various times after plating, NGF was added to the medium of pairs of sister cultures. On day 13, cells were collected from all cultures and assayed in duplicate for CAT. Cell counts confirmed that the cell number in both NGF-treated and untreated cultures did not change significantly during the course of the experiment.

induction of CAT in cultures of sympathetic neurones by non-neuronal cells[3].

The presence of CAT activity in PC12 cells was not only limited to tissue cultures; tumours of PC12 cells grown subcutaneously in rats had CAT activities on the order of 200 pmol min^{-1} mg^{-1}. Furthermore, we have detected CAT activity in tissue from two catecholamine-secreting human pheochromocytomas (unpublished).

Parallel experiments on PC12 cultures revealed that the s.a. of tyrosine hydroxylase (TH), the first enzyme in the synthetic pathway of catecholamines, also increased as a function of cell density (Fig. 1*a*, *b*). Thus, both CAT and TH seem to be similarly inducible in PC12 cultures and to be at maximal levels at high cell density. Such findings lead to the question of whether both neurotransmitter synthesising systems coexist in the same cells. Several pieces of evidence suggest (but do not unequivocally prove) that this is so. First, as judged by histochemical localisation, virtually every PC12 cell in high density cultures contains catecholamines[5,14]. Secondly, ultrastructural studies of PC12 cultures have revealed that dense-core granules (of the type thought to contain catecholamines) and small agranular vesicles (of the type usually considered to contain acetylcholine) are present in the same cells[5,14]. To test further the possibility of dual neurotransmitter synthesis, PC12 cells (250 generations after isolation) were subcloned as previously described[5]. Table 1 reveals that of the single-cell subclones isolated and tested at high cell density, all seven had high levels of TH activity while two also displayed reasonably high levels of CAT activity. This suggests that although the PC12 line is heterogeneous with respect to CAT activity, at least a given subpopulation of cells contains both enzymes. Further experiments will be needed to determine whether such subclones will themselves remain homogenous for CAT activity and whether CAT activity may be induced (either spontaneously or by treatment with conditioned media) in the subclones with low levels of this enzyme.

As mentioned above, PC12 cells respond to NGF by cessation of replication and extension of neurites. To access the effect of NGF treatment on CAT activity in PC12 cells and to dissociate such effects from those on cell division (and thus cell density as well), the following experiment was performed. A stable population of division-arrested PC12 cells was produced by treatment with 1 μM cytosine arabinoside (ara-C) for one week. Such cells were then subcultured at low density (1.2×10^{5} per 35-mm dish) in the presence of ara-C, treated with 2.5 S NGF (ref. 15) for various periods and then harvested and assayed for CAT activity. The ara-C treated cells began to extend neurites within 2 d of treatment with NGF, but did not do so in its absence. The results of this experiment (Fig. 1*c*) revealed that the s.a. of CAT underwent a significant increase within several days of NGF treatment. Furthermore, the time course of this increase roughly paralleled that of neurite outgrowth. No conclusions regarding a causal relationship between the two events could be drawn, however.

Table 1 Enzymatic activities of subclones of PC12 cells

Subclone	Specific enzymatic activity (pmol per min per mg protein) TH	CAT
1A	2,866	11
2B	3,468	170
3A	2,315	15
3B	1,847	8
D2	2,522	153
B1	3,054	19
C2	2,193	14

Single cell subclones were isolated as previously described[5]. Clones were grown to high density (20 generations after initial isolation), harvested and assayed for specific enzymatic activity. Results are means of duplicate assays on first (TH) or first and second (CAT) passage cultures. Lower limit of sensitivity of CAT assay was 0.5 pmol per min per mg protein.

Table 2 Synthesis and storage of ACh by PC12 cultures

	Untreated	NGF-treated
CAT activity		
pmol per min per mg protein	440±17	520±20
pmol per min per 10^6 cells	31±2	83±5
ACh content		
pmol per mg protein	1,335±77	5,594±637
pmol per 10^6 cells	93±7	892±146

Data are mean values (±s.e.m.) based on assays performed in quadruplicate on NGF-treated (22 d in the presence of 50 ng ml^{-1} NGF, 3.5×10^{6} cells per dish) and high density untreated (13.6×10^{6} cells per dish) PC12 cultures.

Experiments were also carried out to assess whether PC12 cells could store the acetylcholine (ACh) they synthesised and whether NGF treatment would affect this capacity. Since PC12 cells (NGF-treated and untreated) contain high levels of cytoplasmic acetylcholinesterase activity (F. Rieger, P. Taylor and L.A.G., in preparation), it is likely that any endogeneous ACh is stored rather than free in the cytoplasm. Accordingly, cultures of NGF-treated and of high-density NGF-untreated PC12 cells with comparable specific activities of CAT were assayed for their contents of ACh by the sensitive radioassay of Goldberg and McCaman[16] (Table 2). Although both types of cultures contained substantial quantities of ACh (nmol per mg protein), those treated with NGF contained three- to fourfold more ACh per mg protein and an average of about tenfold more per cell. One possible basis for this difference in content is that NGF-treated PC12 cells contain many more small agranular vesicles than do untreated cells[5,14].

The data in Table 3 show the results of experiments which demonstrate that PC12 cells can not only synthesise and store ACh, but also release it on exposure to a depolarising medium. While release of ^{3}H-ACh was significantly enhanced in the presence of 51.5 mM K^+, release of ^{3}H-choline was not. In three separate experiments, the proportion of the cellular contents of labelled ACh released by elevated K^+ was between 15 and 20%. We have previously obtained comparable results for the release of noradrenaline from PC12 cells in similar conditions[7]. Thus, it is possible that both neurotransmitters may be secreted by the same cells. Evidence for such a dual release has been presented for rat sympathetic neurons in culture[17]. Finally, as shown for nerve endings and synaptosomes (ref. 18) the K^+-induced increase in release of ACh from PC12 cells was blocked in media either free of Ca^{2+} or containing elevated (10× control) Mg^{2+}.

In conclusion, cultures of the noradrenergic PC12 line of rat pheochromocytoma may synthesise, store and release significant quantities of ACh. The duality of neurotransmitter function shown by this type of cell may resemble, as well as have common origins with, that shown by other neural crest derivatives of normal and neoplastic origin. In this context, PC12 cells should prove useful in the study of various aspects of acetylcholine metabolism as well as the regulation and specification of neurotransmitter properties in developing neurones.

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Table 3 Release of ACh from PC12 cells

Release conditions	d.p.m. released per culture of Choline	ACh
Control	27,750±5,550	6,110±2,830
51.5 mM K$^+$	33,860±2,890	21,650±3,330
51.5 mM K$^+$, 0 Ca^{2+}	28,310±2,220	4,440±2,940
51.1 mM K$^+$, 10×Mg^{2+}	30,530±2,810	11,100±2,330
Intracellular content before release	71,040±6,660	108,225±8,800

Sister cultures (collagen-coated 35-mm dishes containing 2×10^6 cells) of NGF-treated (14 d) PC12 cells were washed free of culture medium and incubated (at 37 °C in this and all subsequent incubations) for 30 min in 1 ml of HEPES-buffered Ringers saline (KRH) (ref. 7) supplemented with (methyl-^{3}H)-choline (10.7 μCi per dish; s.a. = 2.5 Ci mmol^{-1}). The 'loading' medium was then removed and the cultures were washed three times with saline and then maintained for 1 h with 1.5 ml of culture medium RPMI 1640 supplemented with 2% horse serum to allow washout of unbound ^{3}H-choline and its derivatives. Cultures were then pre-incubated for 15 min in KRH before successive 20-min incubations with 1 ml aliquots of KRH (control medium lacking Ca^{2+} or with 10×Mg^{2+} also gave comparable final values) and then KRH modified as described above to contain 51.5 mM K$^+$ and the indicated levels of Ca^{2+} and Mg^{2+}. Eserine (30 μM) was also present during incubations to inhibit cellular acetylcholine esterase. The levels of ^{3}H-choline and ^{3}H-acetylcholine released by and contained within the cells were determined as follows by modifications of previously described procedures[10,16,18,22]. The KRH fractions were placed on ice immediately after the incubations and shortly thereafter extracted with 3 ml of heptan-3-one containing 20 mg ml^{-1} tetraphenylboron. Choline and ACh were then extracted from the organic phase in 1 ml of 0.4 M HCl which was then lyophilised to dryness. Choline and ACh remaining within the cells after release was collected by homogenising in 500 μl of 1 M formic acid–acetone (15:85 v/v). The homogenate was centrifuged at 1000*g* for 5 min and the supernatant was taken to dryness *in vacuo*, resuspended in 1 ml of KRH and then extracted and lyophilised as above. Each dry sample was re-dissolved in 40 μl of chromatography solvent containing authentic carrier choline and ACh (each 10 mM) and 25 μl of this was spotted onto thin layer chromatography (TLC) plates (Eastman no. 6064). After chromatography as previously described[22] the plates were dried and the locations of carrier ACh and choline were identified by exposure to I$_2$ vapour. Each chromatography track was then cut into 5-mm horizontal strips which were placed in separate glass vials and extracted with 1 ml of 0.1 M HCl for 30 min. Liquid scintillation fluid (Instagel-Packard) was added to the vials which were then assayed for radioactivity at a counting efficiency of 23%. Data are presented as radioactivity which co-chromatographed with authentic ACh and choline. Recoveries were estimated on the basis of that of samples of ^{3}H-choline which were similarly processed (ACh has been shown previously to have a comparable recovery to that of choline[18]). Values are given (±s.d.) for an experiment with seven cultures (three for 51.5 mM K$^+$ and two each for 0 Ca^{2+} and 10×Mg^{2+}). Comparable release data were obtained in two other separate experiments.

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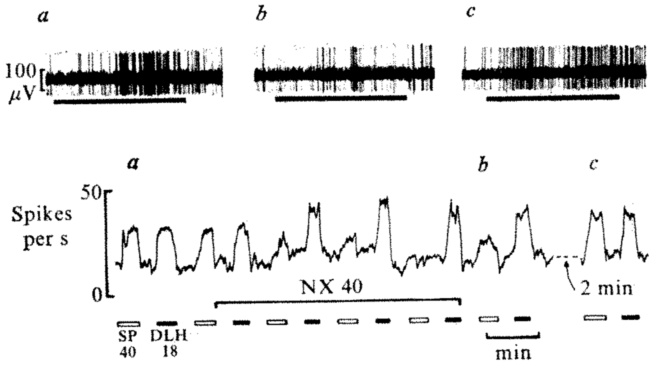
Substance P and opiate receptors

IT has recently been demonstrated that the undecapeptide substance P (SP) produces analgesia in mice when administered intracerebroventricularly (i.v.) or intraperitoneally (i.p.)[1]. This effect of SP was reversed by naloxone indicating the possible involvement of central opiate receptors. The possibility that SP may act on opiate receptors at the single neurone level has been investigated on spinal Renshaw cells in the cat. These cells possess stereo-specific opiate receptor sites[2,3]. The results obtained show that SP excites Renshaw cells and that this action is antagonised by naloxone. Substance P also reduced the excitation of Renshaw cells by acetylcholine.

Experiments were performed in 12 cats anaesthetised with pentobarbitone sodium (35 mg kg^{-1} i.p. initially and supplemented i.v. as required). Blood pressure was continuously monitored and body temperature was maintained at 37 °C. A lumbar laminectomy was performed and the cut central ends of S1 or L7 ventral roots were stimulated to identify Renshaw cells. Extracellular action potentials were recorded *via* the centre barrel (4M NaCl) of a seven barrel micropipette (tip diam. 6 μm). The outer barrels of the micropipette contained aqueous solutions of the following drugs to be ejected electrophoretically: substance P (0.0007 M in 0.165 M NaCl), morphine sulphate (0.05 M in 0.165 M NaCl), naloxone hydrochloride (0.1 M), acetylcholine chloride (0.5 M), DL-homocysteic acid (0.2 M, *p*H 7.2), NaCl (0.165 M).

Substance P excited 17 of 34 Renshaw cells when ejected with cationic currents (40–80 nA, mean 58 nA). Excitation by SP was invariably marked, of rapid onset and comparable to that of DL-homocysteic (see Fig. 1). On, five cells however, the excitatory effects of SP were of slower onset and outlasted the durati on of electrophoretic ejection by 5–20 s. The differences in the time course of SP excitation in the present experiments and the inconsistent effects of SP in other studies[5] may be associated

Fig. 1 Rate meter record (spikes per s against min) illustrating excitation of a Renshaw cell by alternate iontophoretic ejection of substance P (SP 40 nA) and DL-homocysteic (DLH, 18 nA). Ejection of naloxone (NX 40 nA) from an adjacent barrel of the same pipette reversibly reduced SP but not DLH-induced excitation. Extracellular spike records *a*, *b* and *c* of substance P effects correspond to the labelled rate meter response. The duration of iontophoretic ejections is indicated by the horizontal bars below each record. The spike records also show that SP excitations were not accompanied by changes in spike amplitude.



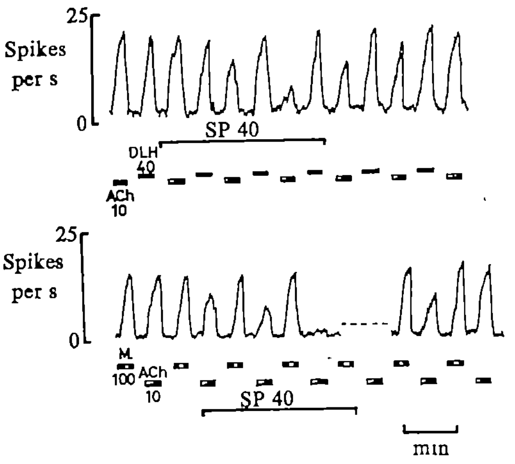


Fig. 2 Rate meter record showing excitation by alternate ejections of acetylcholine (ACh 10 nA) and DLH (40 nA). Prolonged electrophoretic administration of substance P had no effect on the spontaneous firing of this cell but SP (40 nA) reversibly reduced ACh and not DLH responses. The lower record from another Renshaw cell shows that a similar prolonged ejection of SP again reversibly reduced ACh excitation but had little affect, at this current (40 nA), on morphine (100 nA)-induced excitation. Recovery of ACh sensitivity was more prolonged—the dashed line indicates a break of 3 min. A higher current of SP also reduced excitation by morphine on this cell (not shown).

with variable rates of release from individual micropipettes[4,5]. No desensitisation or tachyphylaxis of SP-induced excitation was observed though this phenomena may be a feature accompanying SP excitation in other areas of the CNS (refs 4–8).

Substance P-induced excitation was reversibly reduced by the narcotic antagonist naloxone (30–40 nA, mean 36 nA) on five of the six neurones, whereas the excitatory action of DL-homocysteic was unaffected on three cells and slightly enhanced on the others (Fig. 1). On the same cells, the excitatory effects of morphine and acetylcholine were also reversibly reduced by naloxone in agreement with earlier findings[2,3,9].

In contrast, prolonged ejections of SP with relatively low currents (20–40 nA, mean 30 nA for 2 min or more) markedly reduced the sensitivity of Renshaw cells to acetylcholine while having little effect on spontaneous firing or DL-homocysteic-induced excitation of the same cells (Fig. 2, upper record) This depression of acetylcholine sensitivity was often prolonged (Fig. 2, lower record). Similar results were obtained from 14 other Renshaw cells. This interaction of SP has been noted in another laboratory[10] and we have previously described a similar reduction of acetylcholine sensitivity of Renshaw cells by another peptide, enkephalin[11].

In tests on three cells, SP antagonised both morphine and acetylcholine excitation. However, in each cell the acetylcholine responses were reduced by lower ejecting currents of SP (see Fig. 2). Interestingly, enkephalin also differentiates between excitation induced by morphine and acetylcholine[11].

The present results show that the excitatory effects of SP on Renshaw cells are remarkably similar to the excitatory effects of enkephalin and to the stereospecific effects of morphine[2,3,11]. Additionally, the responses to these three substances are antagonised by naloxone. Thus, these excitatory effects of SP are consistent with an action on opiate receptors. Whether the activation of such receptors on the Renshaw cell is related to the analgesic actions of SP[1] or opiates (see refs 3, 9) is uncertain. However, it may be significant that the regional distribution of SP concentration and opiate receptor binding sites in other brain areas is similar[12,13]. Thus, activation of opiate receptors in these areas might explain the analgesic actions of SP more readily.

The reduction of acetylcholine sensitivity produced by SP might be unrelated to an action at opiate receptors since morphine enhances acetylcholine responses on Renshaw cells[3,9]. However, this action of SP has been demonstrated in several brain areas[5,8] and is shared by other peptides, for example, enkephalin[11] which suggests that polypeptides should be considered to exert a multiplicity of actions at central neurones.

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Evidence for a cyclic nucleotide-mediated calcium flux in motor nerve terminals

WE recently suggested that a cyclic nucleotide system might participate in neuromuscular transmission by regulating the flux of calcium and/or sodium into the nerve ending[1–4]. The purpose of the present investigation was to refine this postulate by studying the interactions between the cyclic nucleotide system and reagents that affect calcium and sodium flux in nerve terminals differentially. Recording from single motor axons of cat soleus nerves *in vivo*, we found that the calcium antagonists verapamil (iproveratril)[4,5] and lanthanum[6] blocked stimulus-bound repetitive action potentials (SBR) produced by the administration of dibutyryl (db) cyclic AMP and sodium fluoride, whereas tetrodotoxin (TTX) did not alter the nerve or muscle response to these reagents. The results suggest that an intraterminal cyclic nucleotide system may regulate calcium flux into the motor nerve ending.

The preparation has been described before[7]. Cats were anaesthetised with 70 mg kg^{-1} α-chloralose and the popliteal fossa was exposed. All branches of the popliteal artery except that which supplies the soleus muscle were occluded and all branches of the tibial nerve except that to the soleus muscle were severed. Both heads of the gastrocnemius muscle were removed and the tendon of the soleus muscle was attached to a strain gauge. A dorsal laminectomy was performed, the ventral root of L7 was cut and its distal stump was split until a strand containing a single functional axon to the soleus muscle was found. This strand was placed over bipolar platinum recording electrodes. A bipolar platinum stimulating electrode was placed on the soleus nerve near its entry into the muscle and supramaximal pulses lasting 0.1 ms were applied every 2.5 s. When appropriate, tetanic stimulations of 400 Hz for 10 s were applied every 5 min. The following materials were injected into the popliteal artery: N^6-2′-O-dibutyryl-adenosine-3′, 5′-monophosphoric acid, cyclic (db cyclic AMP), sodium fluoride (NaF), lanthanum chloride ($LaCl_3$), tetrodotoxin (TTX) and verapamil (a gift from Knoll laboratories). Each compound was dissolved in 0.85% NaCl solution so that 0.1 ml contained the amount to be administered per kg.

Forty injections of verapamil (0.001–200 μg kg^{-1}) were administered to seven animals. Doses lower than 0.01 μg kg^{-1} had no effect on the SBR induced by db cyclic AMP but 0.1 μg kg^{-1} depressed it in all seven preparations (Fig. 1). It completely prevented the production of SBR in five of these and reduced the duration of repetitive activity and the total number of repetitive potentials in the others (8 ± 15% (s.e.) and 6±3% of control, respectively). The administration of higher doses (up to 200 μg kg^{-1}) did not increase the effect of depression; it was impossible to prevent SBR completely in all fibres. Verapamil (0.1 μg kg^{-1}) also reduced the potentiation of muscle force caused by the transmission of the neural SBR to the muscle (20±4% of control) (Fig 1). In addition, verapamil reduced the brief bursts of action potentials in the motor axons and the rapid asynchronous action potentials and contractions in the muscle which were induced by the administration of db cyclic AMP to preparations in which the stimulator was turned off (drug-induced activity, DIA). Recovery from verapamil occurred within 5–15 min.

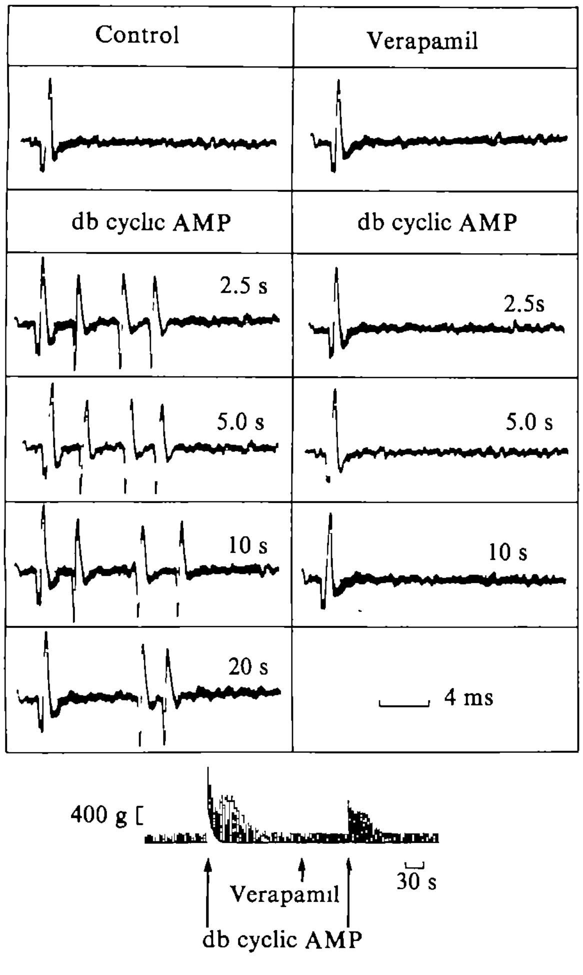

Fig. 1 Top, effect of verapamil on SBR produced in a soleus motor axon by db cyclic AMP. On the left, the first trace shows the response to a single stimulus applied before either drug; subsequent traces show the responses to similar stimuli applied once every 2.5 s after the intra-arterial administration of db cyclic AMP (200 μg kg^{-1}). On the right, the first trace shows the response of a single stimulus applied after the intra-arterial administration of verapamil (0.1 μg kg^{-1}) and subsequent traces show the responses of similar stimuli to db cyclic AMP following verapamil administration. Bottom, effects of verapamil on the potentiation of soleus muscle force produced by db cyclic AMP. Left, response produced by db cyclic AMP (200 μg kg^{-1}) on a neurally-stimulated muscle. Right, response of the same preparation to db cyclic AMP after administration of verapamil (0.1 μg kg^{-1}).

The effects of verapamil on the responses to NaF (100 μg kg^{-1}) were the same as on those to db cyclic AMP. Verapamil (0.1 μg kg^{-1}) blocked SBR in six of eight preparations and significantly reduced the duration of repetitive activity and the total number of repetitive potentials in the others (15±10% and 17±11% of control, respectively). Verapamil also reduced the potentiation by NaF of indirectly evoked muscle contractions (15±6% of control) and diminished the DIA produced by the administration of NaF to unstimulated preparations.

Thirty-five injections of LaCl$_3$ (0.01–100 μg kg^{-1}) were administered to six animals. A dose of 0.01 μg kg^{-1} significantly prevented SBR produced by the administration of db cyclic AMP (200 μg kg^{-1}). It completely prevented the production of SBR in four of these and reduced the duration of repetitive activity and the total number of repetitive potentials in the others (17±9% and 15±8% of control, respectively). Administration of higher doses (up to 100 μg kg^{-1}) did not increase the degree of suppression; it was impossible to prevent SBR in all preparations. LaCl$_3$ (0.01 μg kg^{-1}) also significantly reduced the potentiation of muscle contraction produced by the administration of db cyclic AMP to indirectly-stimulated preparations and diminished DIA. Recovery from LaCl$_3$ occurred within 5–15 min.

The effects of LaCl$_3$ on the responses to NaF (100 μg kg^{-1}) were also the same as on those to db cyclic AMP. LaCl$_3$ (0.01 μg kg^{-1}) blocked SBR in five of seven preparations (Fig. 2) and significantly reduced the duration of SBR and the total number of repetitive potentials in the others (20±12% and 21±12% of control, respectively). LaCl$_3$ also reduced the potentiation of indirectly evoked muscle contractions induced by NaF (37±6% of control) (Fig. 2) and diminished DIA.

Tetrodotoxin (0.01 μg kg^{-1}) was administered to five animals. This dose significantly depressed the repetitive neural activity which follows high frequency stimulation (post-tetanic repetition, PTR); the duration of PTR and the total number of post-tetanic repetitive potentials were reduced to 48±6% and 45±6% of control, respectively and also reduced the potentiation of muscle force caused by the transmission of the neural PTR to the muscle (64±4% of control (Fig. 3). In some cases, this dose of TTX also impaired neuromuscular transmission. Since the generation of PTR is thought to be related to sodium accumulation in the nerve during high frequency stimulation, its reduction by TTX was expected[8-10]. At 0.01 μg kg^{-1}, however, TTX did not significantly alter either the duration of SBR or the total number of repetitive potentials produced by the administration of db cyclic AMP (200 μg kg^{-1}) (121±14% and 103±12% of control, respectively) (Fig. 3). Similarly, the SBR produced by NaF (100 μg kg^{-1}) was unaffected by the administration of TTX; neither the duration of SBR (97±13% of control) nor the number of repetitive potentials (96±7% of control) was changed.

Stimulus-bound repetitive activity is thought to occur when the negative after-potential of the nerve terminal is prolonged for a period longer than the refractory period of the proximal portion of the axon. In this condition, the nerve terminal can act as a current sink for the proximal portion of the axon and re-excite it, thus giving rise to repetitive activity[9,10]. These considerations previously led us to suggest that db cyclic AMP and NaF prolong the after-negativity of action potentials in motor nerve terminals[1-3].

Since cyclic nucleotides have been shown to facilitate the influx of both sodium and calcium into cells[11-14], either ion could account for the SBR observed after the administration db cyclic AMP or NaF. The finding that doses of TTX adequate to reduce the sodium-related PTR did not alter the SBR produced by effects of db cyclic AMP or NaF suggests that sodium flux is not responsible for this latter phenomenon. Its sensitivity to the calcium antagonists lanthanum and verapamil, however, suggests that the SBR which accompanies the administration of db cyclic AMP or NaF is mediated by a calcium current.

Since a prolongation of the negative after-potential of the nerve terminal is implicit in this explanation for the generation of repetitive activity by db cyclic AMP and NaF, there are several mechanisms by which calcium influx would serve this end. With regard to a direct mechanism, the influx of calcium is known to carry a late depolarising current in nerves[15] and prolongation of this current by cyclic AMP could directly affect the afternegativity of the nerve terminal. An indirect mechanism is also possible since it has been shown that inward calcium currents activate late potassium conductance in neurones and that this calcium-dependent potassium conductance produces an after-hyperpolarisation[16-18]. Hyperpolarisation of the nerve terminal can set up conditions wherein the negative after-potential of the invading action potential is prolonged enough to generate SBR[19]. This possibility is given further support by reports that theophylline potentiates the after-hyperpolarisation in bullfrog sympathetic ganglia and that this effect is abolished by the removal of extracellular calcium[20].

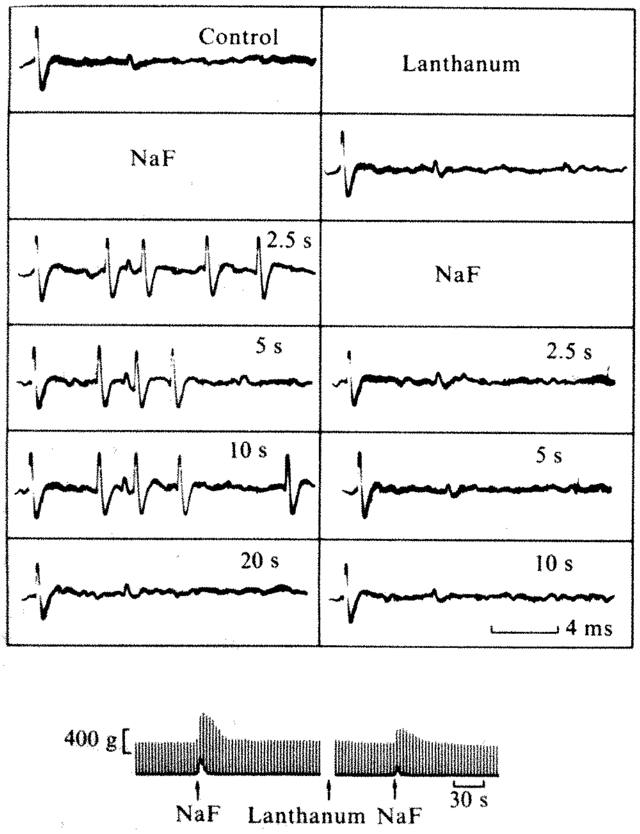

Fig. 2 Top, effect of lanthanum chloride on SBR produced in a soleus motor axon by NaF. Left, first trace is the response to a single stimulus applied before either drug; subsequent traces show the responses to similar stimuli applied once every 2.5 s after the intra-arterial administration of NaF (100 μg kg^{-1}). Right, fiirst trace is the response to a single stimulus applied after lanthanum chloride administration (0.01 μg kg^{-1}); subsequent traces show the responses to NaF following the administration. Bottom, effects of lanthanum chloride on the potentiation soleus muscle force produced by NaF. Left, response produced by the administration of NaF (100 μg kg^{-1}) to neurally-stimulated muscle. Right, response to NaF after the administration of lanthanum (0.01 μg kg^{-1}).

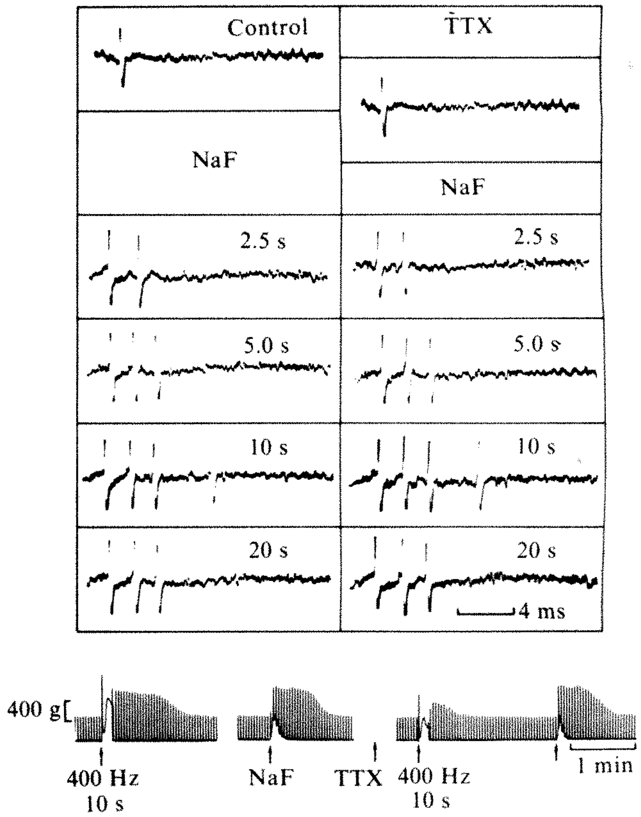

Fig. 3 Top, Effect of TTX on the SBR produced in a soleus motor axon by the administration of db cyclic AMP. On the left, the first trace shows the response to a single stimulus applied before either drug. Subsequent traces show the responses to similar stimuli applied at the noted intervals after the administration of db cyclic AMP (200 μg kg^{-1}). On the right, the first trace shows the response to a single stimulus applied after the intra-arterial administration of TTX; subsequent traces show the responses to db cyclic AMR following the administration of TTX. Bottom, Effects of TTX on PTR and the potentiation of soleus muscle force produced by db cyclic AMP. Left, Responses produced by high frequency stimulation (400 Hz for 10 s) and by administration of db cyclic AMP (200 μg kg^{-1} intra-arterially). Right, Response of the same preparation to high frequency stimulation and db cyclic AMP after administration of TTX (0.01 μg kg^{-1}).

The conclusion that intraneuronal cyclic AMP induces an influx of calcium is also of interest because an influx of calcium into nerve endings is known not only to carry a depolarising current and increase potassium conductance, but also to trigger the release of transmitter[21]. This suggests that cyclic nucleotides might be important in transmitter release.

In summary, our observations indicate that db cyclic AMP and related reagents produce repetitive activity in motor axons by promoting the influx of calcium into the motor nerve terminal and suggest that a cyclic nucleotide system may be part of the mechanism that regulates calcium flux into motor nerve terminals.

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A modifier of $(Na^+ + K^+)$ ATPase in commercial ATP

WHEN most $(Na^+ + K^+)$ATPase preparations are allowed to hydrolyse ATP in the presence of different concentrations of Na^+ and K^+ ions, but with the sum $[Na^+]$ plus $[K^+]$ held constant, the behaviour observed is similar to that shown in the dotted curve of Fig. 1. K^+ ions activate at low concentrations, and inhibit (by competing with Na^+ ions at intracellular sites) at high concentrations. There is a broad plateau in the middle of the curve. We reported[1] recently that $(Na^+ + K^+)$ATPase from the outer medulla of pig kidney showed behaviour of a different kind, like that shown in the solid curve of Fig. 1. Here, K^+ ions apparently inhibit even at relatively low concentrations, so that, as the activating effect of K^+ ions approaches saturation, the inhibitory effect becomes predominant and causes a sharp fall to an intermediate level of activity. This effect was more pronounced at 20 or 30 °C than at 37 °C, and at *p*H 7.5 than at *p*H 6.5. We have now discovered that this peculiar behaviour depends on the use of Sigma 'Sigma grade' ATP, and is not seen with Boehringer ATP (see Fig. 1). We report here that many samples of 'Sigma grade' ATP contain something which reacts with $(Na^+ + K^+)$ATPase reversibly and with a high affinity, making the enzyme much more sensitive to inhibition by K^+ ions. The changed response to K^+ ions, and the sensitivity of this response to *p*H can account for the inhibition of kidney $(Na^+ + K^+)$ATPase by 'Sigma grade' ATP reported by Charney, Silva and Epstein[2], and the altered *p*H optimum reported by Katz and Michell[3].

Evidence that it is the Sigma 'Sigma grade' ATP that carries the modifying agent comes from experiments of two kinds. First an experiment like that of Fig. 1 but using an equimolar mixture of Sigma 'Sigma grade' and Boehringer ATP gave the Sigma pattern. Since the modifier must have been present in the mixture, it is evidently the Sigma pattern that represents the behaviour of the modified enzyme. An alternative approach, using mixtures of Sigma and Boehringer ATP in different proportions, is illustrated in Fig. 2. Here the total ATP concentration was held constant at 3 mM or 6 mM, $[Na^+]$ and $[K^+]$ were fixed at 130 mM and 20 mM respectively, and $[Mg^{2+}]$ was equal to the total ATP concentration. It is important to note that 3 mM ATP was sufficient to saturate the enzyme. The results show that, in these conditions, the activity was highly sensitive to the level of Sigma ATP but was scarcely affected by the level of Boehringer ATP. This implies (1) that it is the Sigma 'Sigma grade' ATP that carries the modifier, and (2) that the modifier is not competitive with ATP. Separate experiments (not shown) indicate that the inhibition by Sigma 'Sigma grade' ATP is reversible and is not affected by changes in the enzyme concentration. The results in Fig. 2 may therefore be used to calculate a 'K_i' for Sigma 'Sigma grade' ATP, which works out as 2.18 mM from the data at 3 mM total ATP and 2.39 mM from the data at 6 mM total ATP. If the modifier is present at a molar concentration of, say, 1% of the Sigma ATP, its K_i must be about 23 μM. The figure of 1% is, of course, entirely arbitrary, but it is clear that, on any likely assumptions, the affinity of the modifier for the enzyme must be high.

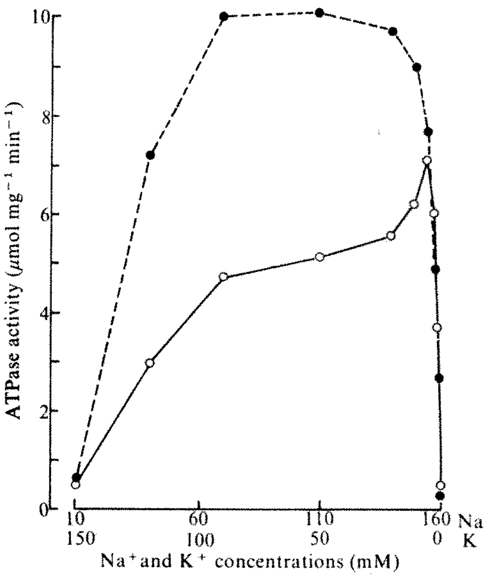

Fig. 1 Rate of ATP hydrolysis as a function of Na^+ and K^+ concentrations. $(Na^+ + K^+)$ATPase prepared from the outer medulla of pig kidney by the simpler method of Jørgensen[6] was incubated at 37 °C for 20 min in a medium containing 2.5 mM ATP, 2.5 mM $MgCl_2$, 30 mM histidine (*p*H 7.5), 0.1 mM EGTA, and NaCl and KCl to give the concentrations shown on the abscissa. The amount of ATP hydrolysed by the end of the incubation did not exceed 10% of the total. The rate of hydrolysis for each set of conditions was estimated from the orthophosphate released after 20 min, as preliminary experiments showed that deviations from linearity during this period could be neglected. ●, Boehringer ATP; ○, 'Sigma grade' ATP.

The most obvious questions raised by these findings are: what is the modifier, and, since Sigma 'Sigma grade' ATP is extracted from horse muscle, does it have a physiological role? The modifier has been present in most, but not all, samples of Sigma 'Sigma grade' ATP that we have used over the past year, and is not restricted to a single lot number. So far we have not succeeded in separating the modifier from the ATP. Passing Sigma 'Sigma grade' ATP down a cation exchange column in the H^+ form did not alter its subsequent behaviour, so that if the modifier is a cation it must be very tightly bound to the ATP. When the ATP was adsorbed on to a column of Dowex 1 resin in the Cl^- form, the modifier was not eluted by a volume of 20 mM NH_4Cl–20 mM HCl sufficient to elute most of the contaminating ADP, but was eluted along with ATP by 250 mM HCl. Clearly, more sophisticated chromatography is necessary. An alternative approach to identifying the modifier is to think of possible candidates and add them individually to 3 mM Boehringer ATP. GTP, CTP, UTP, deoxyATP, adenosine tetraphosphate, 2,3-diphosphoglycerate, fructose 1,6-diphosphate and Ca^{2+} ions have been tested in this way, but none gave the Sigma pattern.

Another interesting question is: what does the modifier do to the enzyme that changes its response to K^+ ions so dramatically? Inhibition by K^+ ions at low concentrations is a feature of the peculiar $(Na^+ + K^+)$ATPase found in low-potassium ungulate red cells, and in these cells the K^+ ions

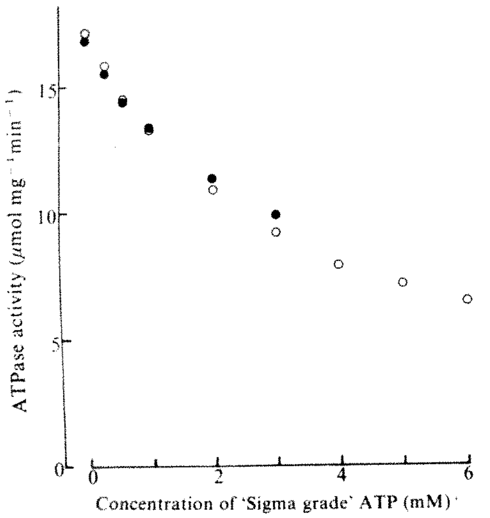

Fig. 2 The effect of substituting Sigma 'Sigma grade' ATP for Boehringer ATP on the rate of ATP hydrolysis by $(Na^+ + K^+)$-ATPase from pig kidney outer medulla. The enzyme was incubated for 20 min at 37 °C in a medium containing 3mM (●) or 6 mM ATP (○), $MgCl_2$ equimolar with the ATP, 20mM KCl, 130 mM NaCl, 30 mM histidine (*p*H 7.5) and 0.1 mM EGTA.

act intracellularly[4]. With kidney $(Na^+ + K^+)$ATPase it is not possible to tell at which surface of the membrane the K^+ ions act. We hope to resolve this problem by using resealed human red cell ghosts, for we have found that the human red cell $(Na^+ + K^+)$ATPase behaves like the solid curve of Fig. 1 when hydrolysis of Sigma 'Sigma grade' ATP containing the modifier is allowed to occur at subnormal temperatures. It is also intriguing that the effect of the modifier on the kidney $(Na^+ + K^+)$ATPase is entirely prevented if the enzyme is first incubated with trypsin in the absence of K^+ ions, until the rapid phase of inactivation (see ref. 5) is complete. The residual enzymatic activity behaves like the dotted curve in Fig. 1 whether Sigma 'Sigma grade' ATP or Boehringer ATP is used (ref. 1 and unpublished).

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The molecular mechanisms of anaesthesia

IT is generally accepted that neutral anaesthetics, such as the *n*-alkanols and *n*-alkanes, act from a hydrophobic site[1]. Discussion has tended to focus on the nature of this site and, particularly, whether it is located in a membrane protein or in the interior of a bilayer[2,3]. If the site is in a bilayer then the question arises as to how the proteins involved in nervous impulse propagation are affected. We present here new evidence concerning the anaesthetic properties of the *n*-alkanes which points to the bilayer as the site of action and also suggests a mechanism for the inhibition of impulse propagation. Briefly, the cut-off in anaesthetic potency on ascending the homologous series is found to be closely related to the decrease in adsorption of the alkane into a cholesterol-containing bilayer. In addition, the anaesthetic hydrocarbons produce a concentration-dependent increase in bilayer thickness. On the basis of these two observations, and from our knowledge of the properties of the ion channel-forming polypeptide gramicidin A (ref. 4) we propose that a thickening of the bilayer regions of the nerve membrane by the alkanes destabilises the open ionic channels formed during electrical excitation.

Several factors make the *n*-alkanes of particular interest in the study of anaesthesia. First, they do not have multiple functional groups which would complicate analyses of their interactions. Second, their interactions with bilayers are relatively well understood from their use as solvents in the formation of black lipid films[5]. Finally, they constitute a homologous series in which there is a hitherto unexplained loss in anaesthetic potency of the higher members[1,6]. This cut-off effect was first revealed by the experiments of Führner[7,8] on general anaesthesia in mice and has been confirmed by Mullins, who showed that mice are unaffected by *n*-decane even after prolonged exposure[6]. We have investigated the ability of *n*-alkanes to block the propagation of the nervous impulse in isolated nerve preparations. Figure 1 shows that *n*-pentane and *n*-hexane cause rapid blockage of the action potential in both squid giant axons and in frog sciatic nerve. Although the information is not strictly required for present purposes, voltage-clamp experiments (to be described elsewhere) show that both the sodium and, to a lesser extent, the potassium currents are reduced by the alkane. For short exposure times and incomplete suppression of the action potential, the blockages were normally reversible. *n*-Nonane and *n*-decane have no significant effect in either preparation. These results are not accounted for by low solubilities and long diffusion times. The new data show, therefore, that as in whole animals, there is a marked decline in anaesthetic potency with increasing chain length in the *n*-alkanes between pentane and nonane.

The adsorption of *n*-alkanes into a lipid bilayer can be measured using black film techniques[5,15]. The bilayers are formed so as to be in equilibrium with solutions of the alkane under examination. Measurements of the electrical capacity per unit area yield the thickness of the hydrocarbon region of the bilayer and, since the areas per molecule of the lipids (for example, phospholipid and cholesterol) present are, at most, weakly dependent on the alkanes used, changes in capacity reflect changes in alkane adsorption. Previous studies have demonstrated an effect of chain length on adsorption, but only for molecules larger than *n*-decane[9]. But, these results were obtained for bilayers which did not contain cholesterol. Analyses of nerve membranes indicate that their cholesterol–phospholipid ratio is approximately 1:3 (ref. 10) and consequently the alkane adsorption experiments were repeated with bilayers of composition comparable with this. Figure 2 shows that the chain length dependence of adsorption is now between *n*-pentane and *n*-decane and, from the thicknesses, it can be concluded that for homologues larger than decane there is very little alkane in the bilayer[9].

The close correlation between the decline in impulse blocking potency and the decrease in bilayer adsorption for the *n*-alkanes, revealed by Figs 1 and 2, would not have been predicted from the bulk phase partition coefficients as in the Meyer–Overton hypothesis[1], and constitutes a simple, if superficial, explanation for the chain length cut-off effect. Moreover, since it is known that the *n*-alkanes accumulate primarily in the centre of a bilayer[11], the correlation reinforces the idea that it is from this position that they exert their anaesthetic action. It is, of course, quite possible that hydrophobic binding sites in membrane proteins may exhibit a size discrimination similar to that of the lecithin–cholesterol bilayer but, until this is demonstrated, the protein site hypothesis is not the simplest available. In a non-equilibrium system it could be argued that even if the protein itself were the site of action and showed no chain length discrimination, a cut-off effect might still be seen if access to the protein were through the lipid bilayer. But, the experimental evidence outlined earlier does not support the notion that the chain length cut-off is a non-equilibrium effect. Hence the route which the anaesthetic might take to a target does not have to be considered.

It may be significant that a similar chain length dependence exists for both impulse blockage within an axon and for general anaesthesia by the alkanes. Thus, even though it seems likely that different proteins are involved in these two processes, they would need to have comparable alkane binding properties. While this is quite conceivable, it is simpler for the moment to invoke the ubiquity of the lipid bilayer.

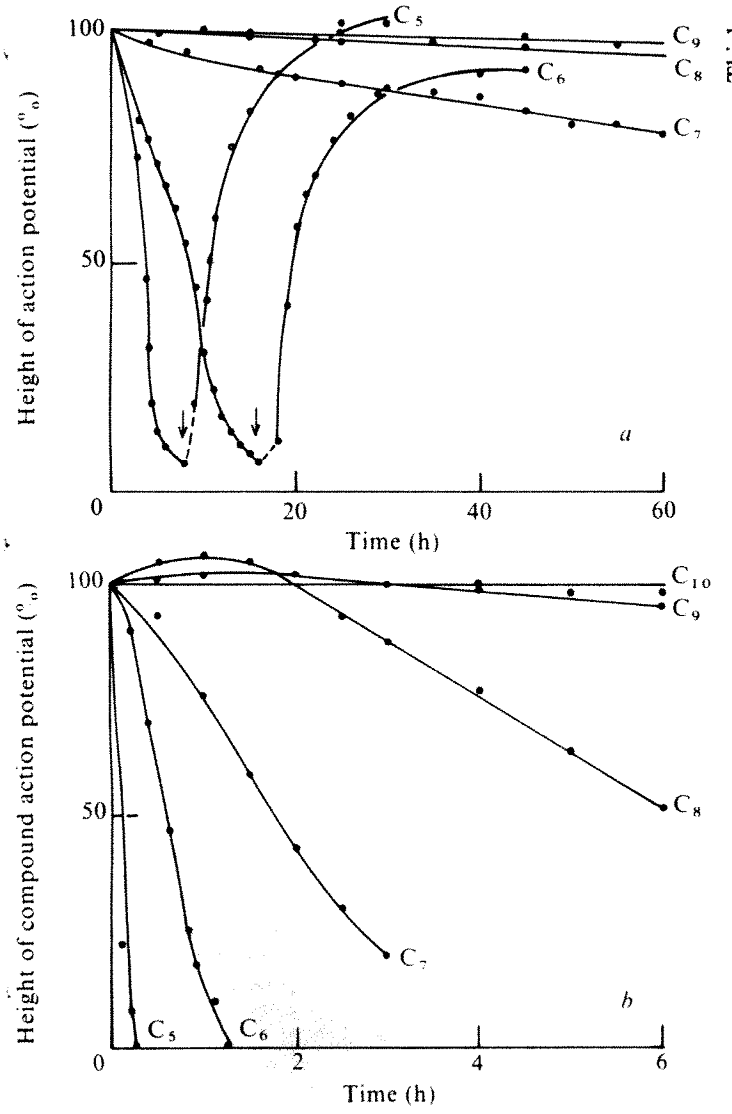

Fig. 1 Nerve impulse blockage by the *n*-alkanes from *n*-pentane (C_5) to *n*-decane (C_{10}). *a*, The propagated action potential of the squid giant axon (from *Dorytheutis plei*) monitored as the chamber was perfused with saturated solutions of the alkanes in artificial seawater. Vertical arrows indicate a switch to alkane-free seawater. Flow rates were approximately 10 ml min^{-1}; internal recording was with a 0.5 M KCl-filled glass capillary; the temperature was 18 °C. The effect of the alkanes on the resting potential of the axon was small and variable. *b*, The compound action potential of the desheathed frog sciatic nerve from *R. temporaria*. Dilute emulsions of alkanes in frog Ringer were used to ensure an adequate supply of material; saturated solutions became depleted by a large uptake into the nerve bundle. Perfusion rates were about 1.5 ml min^{-1}. The nerve was stimulated at 6 Hz and monophasic compound action potentials were recorded from a platinum electrode situated at the end of the nerve. The temperature was 18–21 °C.

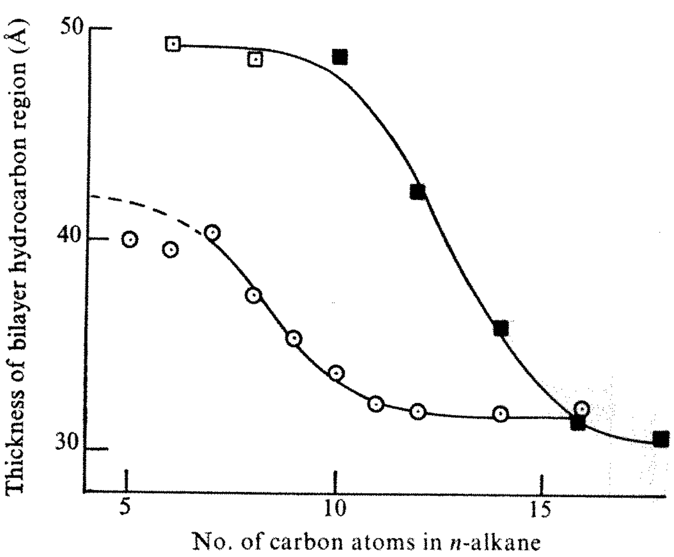

Fig. 2 The variation in hydrocarbon layer thickness of black lipid films formed in equilibrium with different *n*-alkanes. ⊙, Egg phosphatidylcholine–cholesterol (~ 2.5:1) films; the bulk lipid solution was 7 mM phospholipid and 21 mM cholesterol. The pentane and hexane results are possibly low owing to the difficulty of preventing evaporation of these volatile solvents. ⊡, ■, Cholesterol-free egg phosphatidylcholine films. (■, data of ref. 9). The aqueous phase was 0.1 M NaCl and the temperature was 20 °C. In each experiment the lipids were dissolved in the appropriate alkane and the aqueous solution was equilibrated with the lipid solution before the start of the experiment.

The supposition that nerve impulse blockage originates in a lipid bilayer raises the question of exactly how the mechanisms of ion movement involved in electrical excitability might be affected. Recent evidence concerning the unit conductances of ion channels in excitable tissues[12-14] supports the notion that these channels consist of pore-like structures. In view of this, it is of interest to consider how the functioning of a relatively well-characterised polypeptide pore, such as gramicidin A, is affected by modification of its bilayer environment. It is known that the conductance of a black lipid film containing gramicidin decreases rapidly as the thickness and hydrocarbon content of the film increases[4,15]. This arises from a lowering of the stability of the open channels. Since bilayer thickness is increased by the *n*-alkanes (Fig. 2) these observations may have some relevance to anaesthesia. An empirical relationship for the constant K of equilibrium between open and closed gramicidin channels in a black lipid film can be written in the form

$$K \approx A \exp(-h/B)$$

where h is the thickness of the membrane and is considered to take values only larger than or equal to the length of the gramicidin channel. A is a constant, and B, for the particular system and range of h concerned, is 2.2 Å (ref. 15). This equation is consistent with the proposal that a dimpling of the lipid is required for the functioning of a pore in a membrane of thickness greater than the pore length (Fig. 3). The dimpling involves an increase in the surface area of the bilayer and hence requires an investment of energy proportional to the surface tension. On the basis of this crude model, therefore, the free energy of the open pore should be roughly proportional to the bilayer thickness, so giving the observed exponential form of the above equation. In addition, the value of the bilayer tension which corresponds to $B = 2.2$ Å is approximately 3 mN m^{-1}, in good agreement with experiment[9,16].

Provided that the *n*-alkanes increase the thickness of bilayer

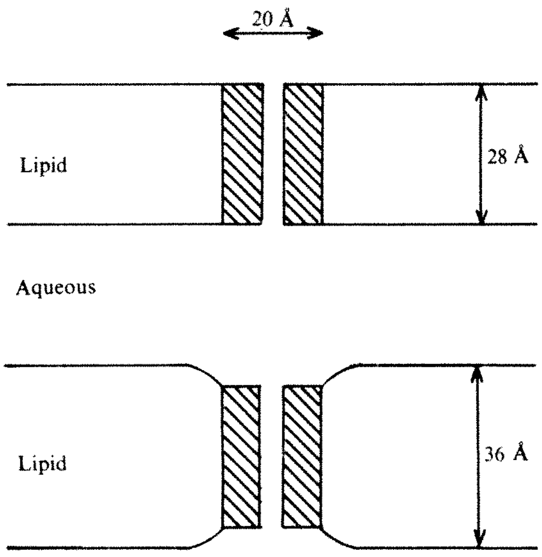

Fig. 3 The thickness–tension model. The shaded region represents a gramicidin channel situated in a lipid bilayer. In the top diagram the bilayer is of thickness equal to the channel length. In the lower diagram the bilayer thickness has been chosen to correspond with the concentration of pentane (about 90% saturated) sufficient to suppress completely the action potential in the nerve axon. The deformation or dimpling of the bilayer in the lower diagram involves a surface free energy change which tends to reduce the stability of the open channel.

regions surrounding the ion channels in excitable membranes, then the type of mechanism proposed for gramicidin could obviously apply also to impulse blockage. Whether or not the channels in an axon are of dimensions comparable with those shown in Fig. 3 is of no great importance for the general argument. Thus, if a channel is normally in equilibrium with the surrounding membrane, a thickness change in the lipid is almost certain to stress the channel and hence destabilise it. While there is no conclusive evidence that the *n*-alkanes do thicken biological membranes, there are strong indications that this is so. First, measurements of the adsorption of *n*-alkanes into erythrocyte membranes have given results which are very similar in both their chain length dependence and in their absolute values to the data of Fig. 2 for cholesterol-containing bilayers (F. Dagger and D.A.H., in preparation). Second, preliminary experiments on the squid giant axon at its normal resting potential, suggest that its capacity per unit area, measured at 1 kHz and with 1 mV applied, decreases by about 15% in pentane-saturated artificial seawater (D.A.H., J. Kimura and B. Urban, unpublished). Circumstantial evidence therefore supports the proposed thickness-tension link between the perturbation of bilayer structure by the alkanes and their anaesthetic potency.

There are no compelling reasons for believing that all anaesthetics share a common mechanism, but it seems possible that the bilayer thickness-tension model outlined above may apply to some more polar anaesthetics. For example, a cut-off in potency with increasing molecular size occurs for the *n*-alkanols[17] and for the barbiturates[18], implying that they also may act through thickening of a bilayer. Furthermore, ethanol and diethyl ether at anaesthetic concentrations both lower the capacity per unit area of black films and therefore must increase the film thickness. Finally, the reversal of anaesthesia by the application of pressure, which has been reported for a range of systems, is thought to take place through the restoration of a hydrophobic region, expanded by the anaesthetic, to its original dimensions[19]. If this is so, it is consistent with the idea of membrane thickness changes and with the existence of a common mechanism for a variety of molecules.

After submitting this paper we learnt of a paper by R. G. Ashcroft, H. G. L. Coster and J. R. Smith (*Nature*, in the press) in which it was reported that benzyl alcohol caused a thickening of lipid bilayers, and in which it was suggested that this effect might be instrumental in inhibiting sodium channel conductance.

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Membrane asymmetry and blood coagulation

ALTHOUGH there is compelling evidence that membrane components are non-randomly distributed between the two surfaces, the physiological significance of membrane asymmetry has so far remained obscure. Since the exterior surface of blood cell membranes is presumably devoid of negatively charged phospholipids which have a regulatory role in blood clotting, a possible function of lipid asymmetry in the coagulation process can be anticipated. We describe here experiments devised to show that the asymmetric distribution of membrane phospholipids in blood cells may serve a biological purpose, by contributing to maintain the delicate balance between regulating haemostasis and avoiding thrombosis.

The main function of phospholipids in blood coagulation is to provide a catalytic surface on which various coagulation factors interact, thus increasing their local concentrations. For example, the final lipid-activated step in the coagulation process is the conversion of prothrombin into thrombin by the prothrombinase complex, which requires at least four components in addition to prothrombin, the proteins factor X_a, and factor V_a, Ca^{2+} and phospholipids[1]. Of these components, only factor X_a is able to convert prothrombin into thrombin by limited proteolysis[1–5], but its individual action is considerably slower than with the complete prothrombinase complex[6].

In situ, the platelet plasma membrane presumably provides the phospholipid–water interface (platelet factor 3), which is required to accelerate the coagulation process[7]. It has been shown[8–10] that the clot-promoting activity of phospholipids *in vitro* is not attributable to a certain phospholipid class, but to a specific negative charge of the phospholipid surface. In particular, negatively charged phospholipids such as phosphatidylserine and phosphatidylglycerol (when properly diluted with neutral phospholipids like lecithin or phosphatidyl ethanolamine) exhibit maximal activation of the coagulation-process[11,12].

It is generally accepted that membranes are highly asymmetric structures both with respect to their proteins and their lipids (see refs 13–16 for recent reviews). We have shown that the non-random distribution of phospholipids between the interior and the exterior half of the platelet plasma membrane is similar to that of the erythrocyte membrane[17]. In both membranes, phosphatidylserine is apparently nearly exclusively located on the cytoplasmic surface, whereas the outer monolayer of the membrane consists of neutral phospholipids, particularly sphingomyelin. The other two major phospholipid classes, lecithin and phosphatidylethanolamine, are present on both membrane sides though not to the same extent. We report here on the possible implications of blood cell membrane asymmetry for the coagulation process. Since methods are readily available to prepare different types of sealed and non-sealed erythrocyte ghosts, this type of membrane has been used in the present study rather than the platelet surface membrane which is more difficult to manipulate.

Blood from healthy volunteers was collected in 0.13 M sodium citrate (9 ml blood + 1 ml citrate). Red cells, non-sealed ghosts and resealed right-side out ghosts were prepared as described previously[18]. Inside-out sealed ghost vesicles were prepared according to Steck[19]. The coagulant activity of these preparations was determined by its ability to shorten the clotting time on recalcification of 'normal' human platelet-poor plasma in the presence of Russell's viper venom (legend to Fig. 1). For the presentation of the coagulant activity of membranes and lipids we report directly the clotting times rather than attempt to convert these to some arbitrary units of activity. It is realised that such clotting times are a complex function of lipid activity, but no real advantage is gained by using conventional log–log calibration curves obtained by serial dilution of plasma. In view of the wide range of the final membrane phospholipid concentration, the values on the abscissa are plotted logarithmically.

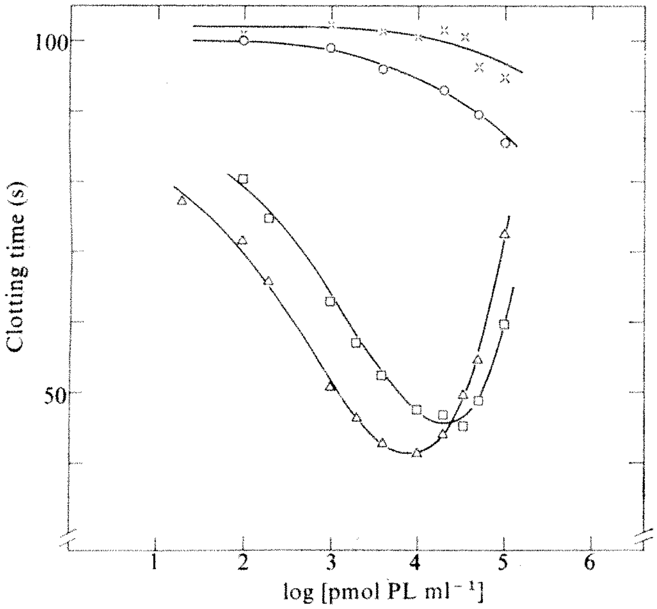

Fig. 1 Effect of in- and outside of erythrocyte membranes on clotting time of plasma. Assay: 0.1 ml of citrated plasma (centrifuged 10 min, 5000*g*) was incubated at 37 °C for 30 s with 12 ng of Russell's viper venom (which gives a blank coagulation time of 100 s on recalcification), followed by addition of 0.1 ml of various dilutions of red cells or ghosts and 0.1 ml 25 mM $CaCl_2$. A stopwatch was started simultaneously with the addition of $CaCl_2$. The time required for the formation of a firm fibrin clot was determined by the arrest of a rotating metal wire (2 mm length, cut from a regular paper clip), driven by a magnetic stirrer. This method gives the same clotting times as observed by using a Kolle hook, but the standard deviations are smaller and usually below 1%. PL, phospholipid added as red cells or ghosts. ×, Erythrocytes; ○, resealed ghosts; △, inside-out vesicles; □ non-sealed ghosts.

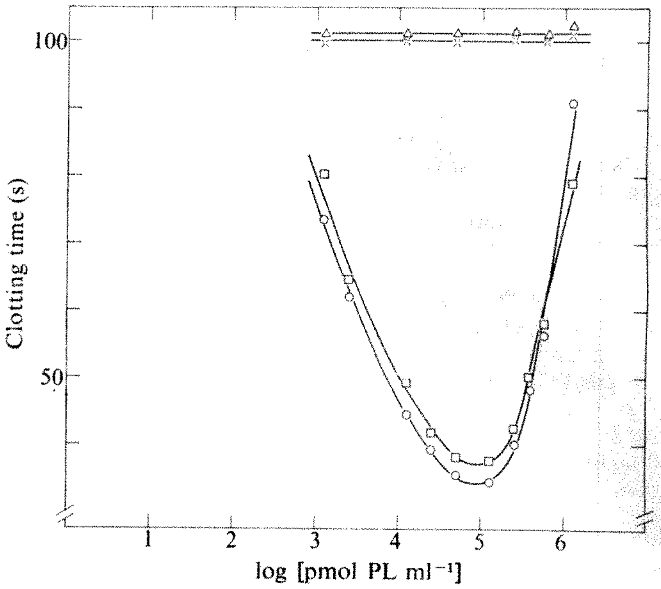

Fig. 2 Effect of liposomes on clotting time of plasma. Liposomes simulate inner and outer half of red cell and platelet plasma membranes. ×, Platelet surface membrane outer phospholipids; □, platelet surface membrane inner phospholipids; △, red cell membrane outer phospholipids; ○, red cell membrane inner phospholipids. For phospholipid compositions see Table 1. Assay conditions as for Fig. 1. PL, phospholipid added as liposomes.

When only the outside of the erythrocyte membrane is exposed (either intact cells or resealed ghosts) no significant reduction of the blank clotting time is observed over a wide range of concentration (Fig. 1). On the other hand, when either the membrane interior (inside-out vesicles) or both membrane sides (non-sealed ghosts) are exposed, a two- to threefold reduction of the coagulation time is observed at optimal concentrations. At higher ghost concentrations, clotting times increase; this is consistent with the view that excess of lipid will result in a dilution of coagulation factors at the lipid surface[20]. The results suggest that only the phospholipids at the membrane interior have procoagulant activity, also since inside-out ghost vesicles are slightly more active that non-sealed ghosts.

This hypothesis was further investigated by testing liposomes with the same phospholipid composition as present in the outer half and inner half of human red cell membranes and pig platelet plasma membranes (Table 1). Handshaken liposomes (4 µmol phospholipid per ml of 0.9% (w/v) NaCl) were used

Table 1 Mole ratios of phospholipid in liposomes simulating outer and inner half of red cell and platelet plasma membranes

	Red cell membrane		Platelet plasma membrane	
	Outer half	Inner half	Outer half	Inner half
PC	44	15	26	34
Sph	44	10	52	4
PE	12	47	20	28
PS	0	28	2	34

Data calculated from refs 17 and 21. PC, phosphatidylcholine prepared from egg yolk[22]; Sph, sphingomyelin prepared from bovine brain[23]; PE, phosphatidylethanolamine prepared from egg yolk[22]; PS, phosphatidylserine prepared from pig brain[24].

rather than sonicated vesicles, to avoid artifically-induced phospholipid asymmetry which is known to occur in mixed vesicles as a result of charge differences and packing properties[25]. It should be noted, however, that with multilamellar liposomes only 10–15% of the lipids are available at the outer surface[26]. As shown in Fig. 2, liposomes having the same phospholipid composition as present in the outer membrane half of either red cell membranes or platelet plasma membranes do not reduce whatever the blank clotting time. On the other hand, liposomes with the same phospholipid composition as the inner half of red cell or platelet plasma membranes produce a threefold reduction of the coagulation time at optimal concentrations. The amount of lipid required is higher than with ghosts, since only a fraction of the total lipid is available at the surface of handshaken liposomes.

It is realised that the measured procoagulant activity of the lipids is not performed at one particular transitory stage of the blood clotting process, in spite of the presence of Russell's viper venom which is known to activate both factor *X* and factor *V* (ref. 27). Nevertheless, the results strongly suggest that the outer surface of blood cells is devoid of phospholipids which are active in blood coagulation, mainly due to the absence of any significant amount of phosphatidylserine. This phospholipid comprises about 30% of the phospholipids at the membrane interior, representing a concentration which has been shown to be optimal in activating prothrombin by the prothrombinase complex[11].

The presence of procoagulant phospholipids only at cytoplasmic surfaces of blood cells is not only consistent with phospholipid asymmetry in membranes, but might also represent an important mechanism both in avoiding thrombosis and in regulating haemostasis. It could be judged as potentially dangerous to have procoagulant phospholipids at the outer surface of blood cells, which may bring about a 'permanent' condition of hypercoagulability. This fact itself could provide a physiological reason for an asymmetric phospholipid distribution in blood cell membranes. On the other hand, after disruption of the vessel wall the sequence of platelet adhesion, release reaction and ADP-induced platelet aggregation, presumably accounts for the primary arrest of bleeding. Provided that platelet factor 3 activity becomes accessable during this process, its availability should come after the release reaction since no phosphatidylserine can be labelled from the outside during the release reaction[28]. Moreover, it has been demonstrated that only a small fraction of lipid procoagulant activity becomes available during release reaction as compared with the activity obtained upon lysis[29,30]. This raises the question whether during or after formation of the primary haemostatic platelet plug, either (partial) lysis of platelets or an as yet unknown mechanism which translocates phosphatidylserine through the plasma membrane is necessary to provide a catalytic lipid surface for interacting coagulation factors.

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Ion transport and rotation of bacterial flagella

BACTERIA swim by rotating their flagella[1–4]. A flagellum consists of a thin helical filament which is joined by a hook to a rod and a set of rings embedded in the cell wall and the plasma membrane[5,6]. Gram-positive bacteria such as *Bacillus subtilis* have two rings, one of them, the M ring, binding to the plasma membrane and the other, the S ring, being located on the outside of the membrane. Gram-negative bacteria such as *Escherichia coli* have two additional rings which are associated with the peptidoglycan layer and the outer lipopolysaccharide membrane. The energy source of the flagellar motor seems to be not ATP itself but rather an intermediate in oxidative phosphorylation which is interpreted as an electrochemical potential gradient for hydrogen ions across the plasma membrane[7–9]. Berg[3,4] has proposed that the rotation of the flagellum is driven by the translocation of ions down an electrochemical gradient. This hypothesis, however, has never been formulated in terms of a specific model. An entirely different mechanism based on hydrodynamic interactions between plasma membrane and basal body of the flagellum has been suggested by Adam[10]. Here, we examine Berg's hypothesis more closely and propose a model for the electromechanical coupling between ion flow and flagellar rotation.

Following Berg[4] we assume that the S ring is attached to the peptidoglycan layer of the cell wall and that the M ring, which is mounted rigidly on the rod, is able to rotate in the plasma membrane (Fig. 1). We further assume that hydrogen ions which have in the extracellular medium a high electrochemical potential with respect to the cytoplasm[11–13] are able to flow through the interstice between the S and M rings and through the periphery of the M ring to the cytoplasm, as shown in Fig. 1. How can this flow of ions generate a torque?

According to current concepts, the passage of ions through cellular membranes takes place within localised structures termed ion channels. An ion channel may consist of a series of polar ligand groups which partly replace the primary hydration shell surrounding the ion in water. In this way the channel offers to the ion an energetically favourable pathway through the hydrophobic interior of the membrane. A well studied example is the gramicidin channel[14–16], which is selective for univalent cations and in which the permeating ion interacts with the carbonyl oxygens of amide groups. In the following we shall assume that the face of the S ring, which is in contact with the M ring, contains radially arranged rows of ligand groups (Fig. 2). If the opposite contact face of the M ring contains similar rows of ligand groups, but arranged in a nonradial direction, the two sets of rows intersect at a certain angle. Evidence for a ring structure with non-radial arrangement

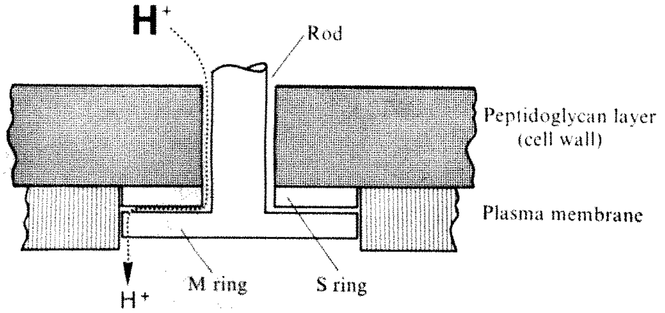


Fig. 1 Structure of the basal body of the flagellum of Gram-positive bacteria, according to De Pamphilis and Adler[5] and to Berg[4]. The S ring is attached to the cell wall and the M ring, which is mounted rigidly on the rod, is able to rotate in the plasma membrane. Hydrogen ions are assumed to flow down an electrochemical gradient from the outside through the interstice between S and M ring and through the periphery of the M ring to the cytoplasm.

of subunits has been obtained by De Pamphilis and Adler[5] in their electron microscopic studies of bacterial flagella, although it was not possible to assign the observed non-radial ring structure to one specific type of ring (M, S, P or L).

Figure 2 shows that, when the S ring is held fixed and the M ring is rotated, the points of intersection move radially with respect to the S ring. We further assumed that the energy of interaction of the ion with one ligand row is not sufficient to compensate for the energy required to remove the ion from water but that an energetically favourable state is reached if the ion is located at the site of intersection where it has twice as many ligand groups available. In these circumstances the ion is constrained to move together with the site of intersection. In the model depicted in Figs 1 and 2, part of the electrochemical gradient of H^+ drops across the rings in a radial direction so that an ion located between the S and M rings experiences a radial (centrifugal) force. As we have assumed that ions are confined to the intersection sites between the two sets of rows, an ion movement down the radial potential gradient results in a rotation of the M ring with respect to the S ring. In this way, ions entering the ring system near the axis and leaving it at the periphery, drive the rotation of the flagellum in a manner not unlike the action of a turbine.

If K is the radial force acting on the ion and θ the angle between the two intersecting ligand rows (Fig. 2), the torque exerted by an ion on the M ring is equal to $(a/2)K \sin 2\theta$, where a is the distance of the intersection point to the centre. The difference in electrochemical potential of H^+ between cytoplasm and extracellular space may be expressed as $e_0\Delta\psi_{eq}$ where e_0 is the charge of the proton and $\Delta\psi_{eq} = \Delta\psi - (59 \text{ mV}) \Delta pH$ is the equivalent electrical potential difference ($\Delta\psi$ is the membrane potential). If the fraction α of $\Delta\psi_{eq}$ acts across the width $(R_2 - R_1)$ of the rings (R_1 and R_2 are the inner and the outer diameter of the rings, respectively) and if the turbine contains on the average n hydrogen ions, then the mean torque M (averaged over one repetition period) is given by

$$M = \alpha B n e_0 \Delta\psi_{eq}$$

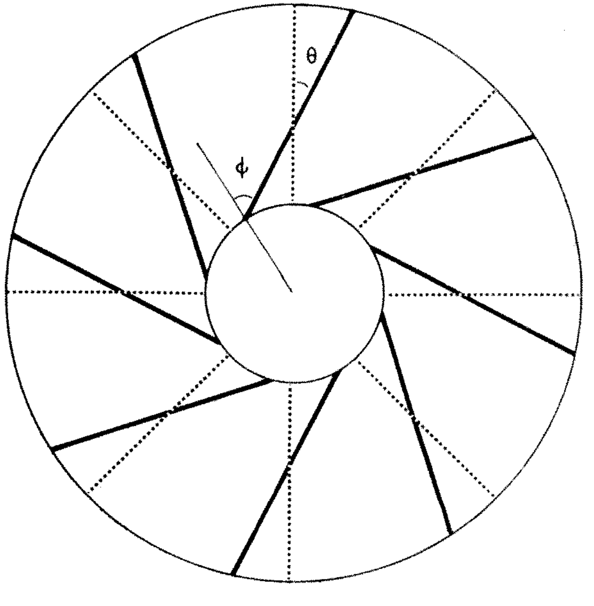
where B is a dimensionless quantity which depends on the geometry of the ligand system. It may be shown that for the model represented in Fig. 2 the relation $B = \rho \sin^2\psi/(\varphi - \text{arc sin} (\rho \sin\varphi))$ holds, where $\rho = R_1/R_2$ and φ is the tilt angle of the non-radial ligand row (Fig. 2). From the structural data of the basal body[5] a value of $\rho \simeq 0.3$ is obtained. If the tilt angle is chosen to be $\varphi = 60°$, one finds that $B \simeq 0.3$. Numerous attempts have been made to determine the electrochemical gradient for H^+ across the bacterial plasma membrane, and there is now considerable evidence that in respiring bacteria $\Delta\psi_{eq}$ is of the order of 0.1–0.2 V (cytoplasmatic side negative)[11–13]. Using these values of $\Delta\psi_{eq}$ and B, and assuming, for the purpose of an estimate to the order of magnitude, $\alpha \simeq 1$ and $n \simeq 10$, one obtains a torque of $M \simeq 10^{-19} J$. This figure may be compared with the actual torque generated by the flagellar motor. From a hydrodynamic analysis of flagellar motion, Coakley and Holwill[17] estimated that the power dissipation of a single flagellum rotating at a frequency of 50 Hz is about $P = 4 \times 10^{-17}$ W. This corresponds to a torque $M = P/\Omega$ of $1.3 \times 10^{-19} J$ (Ω is the angular velocity). Similar values of M were obtained from experiments with tethered bacteria described by Silverman and Simon[2] and by Berg[3]. Thus, the mechanism considered above is able to generate a torque of the required order of magnitude. The predicted torque M is independent of angular velocity Ω and the external load which means that $\Omega = M/f$ should decrease when the friction coefficient f is increased. This is consistent with the experimental findings of Berg[3] who observed that the rotational frequency of tethered cells was approximately inversely proportional to the viscosity of the external medium (see also Schneider and Doetsch[24]).

The number of ions flowing through the turbine in one second may be estimated to be $P/e_0\Delta\psi_{eq} \simeq 4 \times 10^{-17}/1.6 \times 10^{-19} \times 0.2 \simeq 10^3$ (an estimate of 10^3 ions per cycle has been given by Berg[3] for a tethered cell spinning at 10 Hz). If the number ν of ligand rows on each ring is assumed to be identical with the number of subunits found in the electron microscopic study of De Pamphilis and Adler[5] ($\nu = 16$), the rate of ion flow along one row is of the order of 100 s^{-1}. If a single row contains about 10 ion-binding sites then the required jump rate of the ion between adjacent sites is $\sim 10^3 \text{s}^{-1}$. This value is well within the range of feasible jump rates in ion channels[18].

In recent years, it has become clear that bacterial chemotaxis is based on the ability of bacteria to reverse the direction of rotation of their flagella in response to sensory stimuli[19,20]. A reversal of the direction of rotation could occur by a conformational transition of the subunits of the M ring[10], which would change the tilt angle of the ligand rows from φ to $-\varphi$ (Fig. 2). Such a conformational transition could be triggered by a transient change in membrane potential[21–23].

Several modifications of the proposed model are conceivable. Other ion species besides H^+ which have a non-equilibrium distribution across the cytoplasmatic membrane may drive the flagellar motor. Furthermore, the geometry of the ligand rows depicted in Fig. 2 is certainly not optimal and many other

Fig. 2 Hypothetical arrangement of ligand groups on the S and M rings. Ligand rows on the S (dashed) and M rings (full lines) are superimposed. When the M ring is rotated the points of intersection, which are assumed to be ion-binding sites, move radially with respected to the S ring.



arrangements are feasible. Another (less likely) alternative would consist in assuming that the torque is generated between the rod and the bushing in the cell wall, the rod being equipped with straight axial and the bush with helical ligand rows (or vice versa).

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Analysis of positional information applied to cirral patterns of the ciliate *Euplotes*

IN studying the relative positions of structural features on the surfaces of organisms, most biologists are content with qualitative impressions of pattern. This approach is inadequate when there are large variations among individuals, as occurs in the taxonomically important positional patterns formed by the locomotory organelles, the cirri, of the hypotrich ciliate genus *Euplotes*. The frequency distribution of all intercirral distances provides a quantitative measure of cirral pattern that incorporates individual variations. A comparative study[1] among different *Euplotes* populations of such distributions reveals a small set of basic cirral patterns. It also demonstrates that certain cirral patterns may be simulated by morphogenetically plausible alterations in others, thus supporting the hypothesis that certain cirral patterns are derivative of others. The theoretical creation of artificial positional patterns that mimic naturally occurring patterns provides a useful perspective on the systematics of this group of organisms.

The ventral surface of *Euplotes* (Fig. 1) bears a system of morphogenetically unified landmarks, the frontoventral and transverse cirri, which arise during asexual division from subpellicular kinetosomal plaques. These compound ciliary organelles develop from the simultaneous elongation and condensation of a series of five anteroposterior kinetosomal streaks[2]. The posterior portions give rise to the five transverse cirri found universally throughout the genus. Anteriorly, at most two additional cirri develop from each streak, forming the frontoventral cirri. It is the number and relative positions of cirri along the streaks that determine the cirral pattern. In most of the 51 recognised species[3], there are 10 frontoventral cirri, as in *E. harpa* (Fig. 1), with a 'cirrotype' of 10. But some species are of lesser cirrotype, with several common freshwater forms being of cirrotype 9, and a few marine species of cirrotypes 8 and 7.

A simple measure of cirral pattern is obtained by calculating, for each specimen of a sample, from coordinate data for the positions of the frontoventral and transverse cirri (including the buccal cirrus), all intercirral distances. These distances are scaled by a factor external to the cirral system proper, the length of the specimen. For any given population ($n \geqslant 25$) of *Euplotes*, regardless of cirrotype or mean size, the frequency distribution of such relative intercirral distances provides a measure of the cirral pattern of that population. The distributional profiles of different populations then may be compared using the maximum difference ($D_{\max}$) between their cumulative frequency distributions, that is, the Kolmogorov–Smirnov statistic[4].

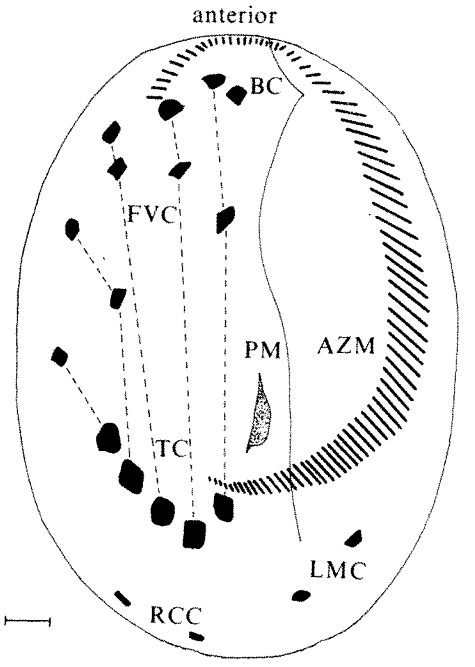

Fig. 1 Schematic diagram of the ventral surface of a silver-stained specimen of *E. harpa*, a brackish-water species with cirrotype 10, illustrating the basal plaques of the frontoventral (FVC) and transverse (TC) cirri as well as the longitudinal subpellicular streaks (dashed lines) from which these cirri arise during morphogenesis. Also illustrated are the buccal cirrus (BC), which is treated as part of the preceding system; the adoral zone of compound ciliary membranelles (AZM) that line the mouth; the right caudal cirri (RCC); the left marginal cirri (LMC), and the paroral membrane (PM). The line scale indicates 10 μm.

To assess the extent of inherent variability in cirral patterns, 17 subclonal populations of the largest species in the genus, the brackish-water form *E. harpa*, were grown in different conditions of temperature and salinity during 3 yr. Samples of at least 50 specimens were selected from each population. All specimens were selected randomly from preparations silver-stained by the Chatton–Lwoff procedure[5], except that only non-reorganising, well impregnated and properly oriented specimens in micronuclear interphase[6] were chosen. Figure 2 shows the relative frequency distributions for the two most dissimilar samples from these populations, all of which were derived from the same original individual. For these two distributions, $D_{\max}$ is 0.0693, and this value provides a conservative criterion for judging the similarities of other distributions. If two samples of, for example, different species differ in their distributions by less than the difference that can exist among subclones of *E. harpa*, it would be unwise to consider their cirral patterns to be different.

By this criterion, many of the numerous species of *Euplotes* have indistinguishable cirral patterns[1]. Most cirrotype 10 patterns are very similar, with only two of 13 geographically disparate samples differing slightly from the common pattern (Fig. 1). Indeed, for each cirrotype known to exist within the genus, at most two cirral patterns exist, as judged by comparisons of the distributions of 33 clonal samples of diverse origin. The internal integrity of each of these few patterns permits the following interpretation of cirral pattern diversity within the genus.

There are only two ways in which a *Euplotes* may possess a cirrotype of 9. The first, as in *E. patella*, involves the absence of a cirrus at the position in which the third cirrus (numbering from posterior to anterior) in the fourth streak (numbering from the organism's left to right), cirrus 3/IV, occurs in *Euplotes* of cirrotype 10. The second variant, as in *E. affinis*, occurs as a result of the absence of the 2/IV cirral position. That is, there are only two cirri in streak IV in forms of cirrotype 9. In the more common variant, the second cirrus occurs at the anterior end of the streak, while in the rarer variant, it occurs in the middle. Results of a distributional analysis based on 16 samples confirm the existence of these two distinct patterns[7] and indicate that the latter variant is the more homogeneous cirral pattern; nonetheless, the more common pattern is not more variable than the subclones of *E. harpa*.

The integrity of each of the above cirral patterns suggests that the cirral patterns of forms with cirrotypes less than 10 can be explained by a simple loss of cirri (either by the direct depletion of kinetosomal numbers in the affected streaks or by the redistribution during morphogenesis of unchanged numbers of kinetosomes). To test this hypothesis, several artificial distributions were constructed by the recalculation of intercirral distance distributions after the removal of the coordinate data for appropriate cirri from samples of *Euplotes* of cirrotype 10. Then these were compared with the distributions of forms with cirral 'deficiencies' presumed to be homologous with the artificial forms.

Figure 3 illustrates the distributions for two freshwater samples of *Euplotes* of cirrotype 9 with the *affinis*-type cirral pattern and the artificial distribution obtained subsequent to the removal of cirrus 2/IV from a freshwater sample of *Euplotes* of cirrotype 10. The fit is remarkably good ($D_{max} = 0.0424$) and well within the range of variation of subclones of *E. harpa*. An adequate fit ($D_{max} = 0.0612$) to a *patella*-type cirral pattern was also obtained from this sample after the deletion of cirrus 3/IV. Similarly, reasonable simulations of both cirrotype 8 and both cirrotype 7 patterns were obtained using artificial distributions derived from the small marine species *E. minuta* (Fig. 4).

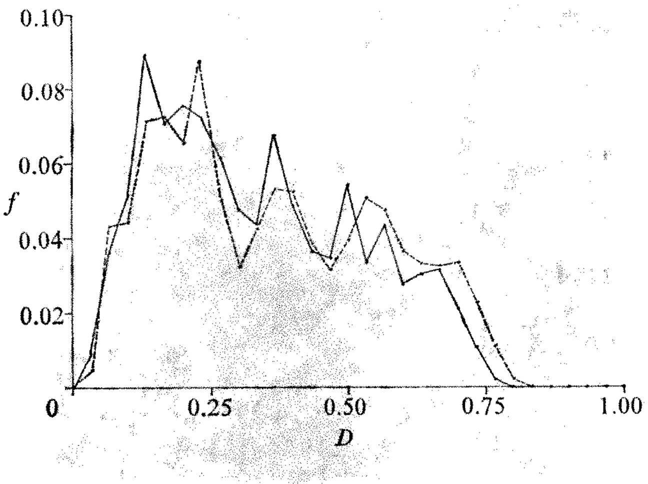

Fig. 2 Relative frequency (f) distributions of scaled intercirral distances (D) for the two most dissimilar ($D_{max} = 0.0693$) of 17 *E. harpa* subclones, based on samples of 57 (dashed line) and 55 (solid line) specimens. Coordinate data for each specimen were obtained by placing a Leitz Ortholux microscope with drawing tube on top of an electronic two-dimensional digitiser. Data were recorded on cassette tape, transferred to nine-track magnetic tape, and thence to on-line disk storage for processing on an IBM 370-165-II computer system, using a Fortran program written by me. For each of the 2,122 specimens, the coordinate data were visually checked by inspection of computer plots. Intercirral distances and their distributions were calculated from the coordinate data, Gould plots were made, and comparisons among distributions were performed automatically.

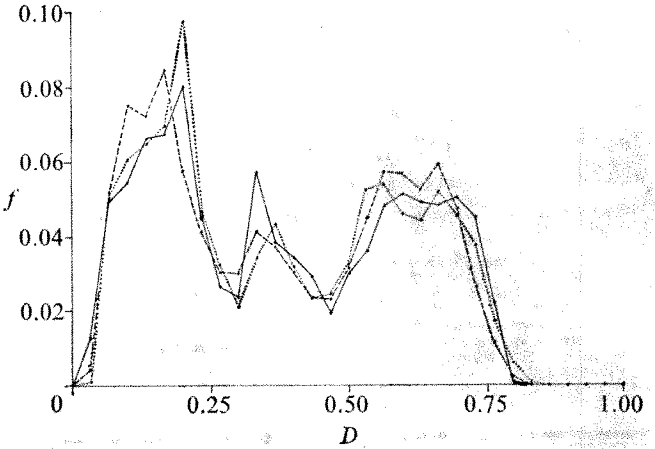

Fig. 3 Relative frequency distributions for two samples of *Euplotes* with cirrotype 8: *E. affinis* from Stevenage, Hertfordshire, UK (dashed line), and from Toronto, Ontario, Canada (dotted line); and an artificial *affinis*-type sample (solid line) created from a sample of *Euplotes* with cirrotype 10 from Inuvik, Northwest Territories, Canada, by the removal of the coordinate data for two cirri.

These results demonstrate the plausibility of explaining certain cirral patterns as derivative from others. In conjunction with quantitative studies of variation in other taxonomic features[8], it is possible to suggest a phylogeny for the genus *Euplotes* that eschews untested typological dichotomies[1].

The analysis of positional data by means of the distributions of interpositional distance is an approach to biological pattern analysis that has obvious applications. Not only may it be applied to the systematics of other hypotrich genera, in which cirri exist in fixed patterns, and to, for example, setal positions in various insects, but it also may be used to study the changes in positional relationships that occur during development in various taxa. It may be possible to show, for example, that certain patterns are arrested stages in the development of others, as is undoubtedly the case with some of the oxytrichid hypotrichs[9] among the ciliated protozoa.

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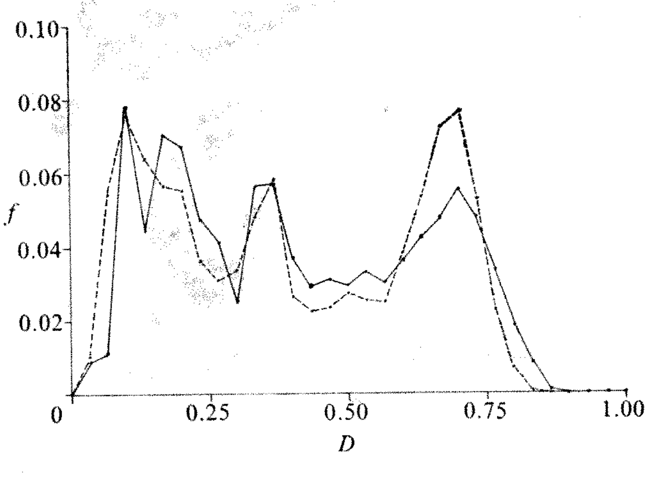
Fig. 4 Relative frequency distributions for an *E. raikovi* sample (dashed line) from Rye Harbor, New Hampshire, and an artificial sample with cirrotype 7 (solid line) created from a sample with cirrotype 10, *E. minuta*, from Villefranche-sur-Mer, France, by removal of the coordinate data for 2/IV, 2/II and 2/I cirri ($D_{max} = 0.0618$).



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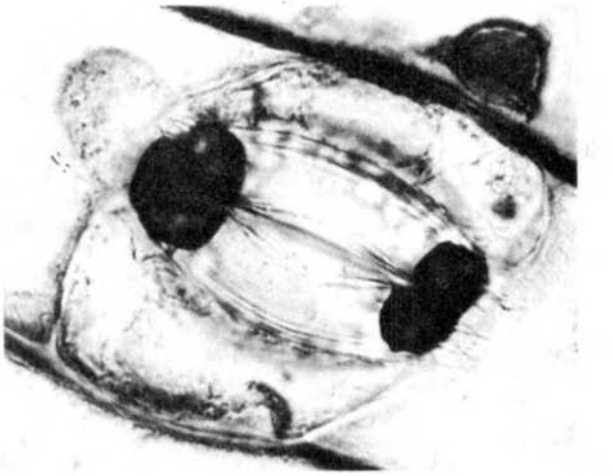
Ion-adsorbent substomatal structures in *Tradescantia pallidus*

STOMATAL movements result from turgor imbalances occurring within the stomatal complex which are largely brought about by differential potassium distribution between the guard cells and adjacent epidermal cells[1-4]. The guard cell vacuole has been established as the sink of the potassium flux during stomatal opening[5,6], but the source of the ions has not been identified. It is generally believed that the ion flux is apoplastic because plasmodesmata have only occasionally been observed between the guard and adjacent epidermal cells[7-9]. We have described substomatal ion-adsorbent sites in some species of filicopsid ferns and Commelinaceae which we believe could act as the immediate sink/source of potassium ions involved in stomatal closing and opening[11]. Discrete accumulations of Macallum's stain for potassium[12] were demonstrated at both poles of the guard cell complex on the face adjacent to the mesophyll. These sites corresponded to substomatal endocuticular swellings observed by scanning electron microscopy which, when collapsed, revealed the presence of a disk-shaped structure on the outer face of the guard cell walls at the site of ion adsorption. Direct observation of the ion-adsorbent sites was prevented by the overlying endocuticular substomatal sacs. We have now observed these sites directly in *Tradescantia pallidus* and obtained evidence which strongly implicates them in stomatal functioning.

Tradescantia pallidus was selected as a more suitable material for further investigations into the form and function of substomatal ion-adsorbent sites because its stomata respond readily to physiological experimentation and it has well developed ion-adsorbent sites, as determined by the Macallum technique[11] (Fig. 1). Segments of fresh whole leaves were mounted lengthwise on a scanning electron microscope stub and cut longitudinally, along the longitudinal axes of the stomata, immediately before being plunged into liquid nitrogen. The specimen was then mounted and observed on the cryostage of a JSM-35 scanning electron microscope in an uncoated condition.

Fig. 1 Stomatal complex of *Tradescantia pallidus* stained for potassium showing the polar accumulations (× 1,000).



Our findings indicate that the ion-adsorbent sites in this species consist of discrete subtetrahedral bodies lying in the intercellular space between the pole of the guard cell and the adjacent polar subsidiary cell (Fig. 2*a*). The ion-adsorbent bodies are closely paired so that each pole of each guard cell is associated with its own single body. While one face of the ion-adsorbent body is adpressed onto the subsidiary cell wall, there seem to be no direct connections between the body and the protoplasts of either the guard or subsidiary cells. Each body has an approximate volume of 0.28×10^{-15} m^3 in comparison

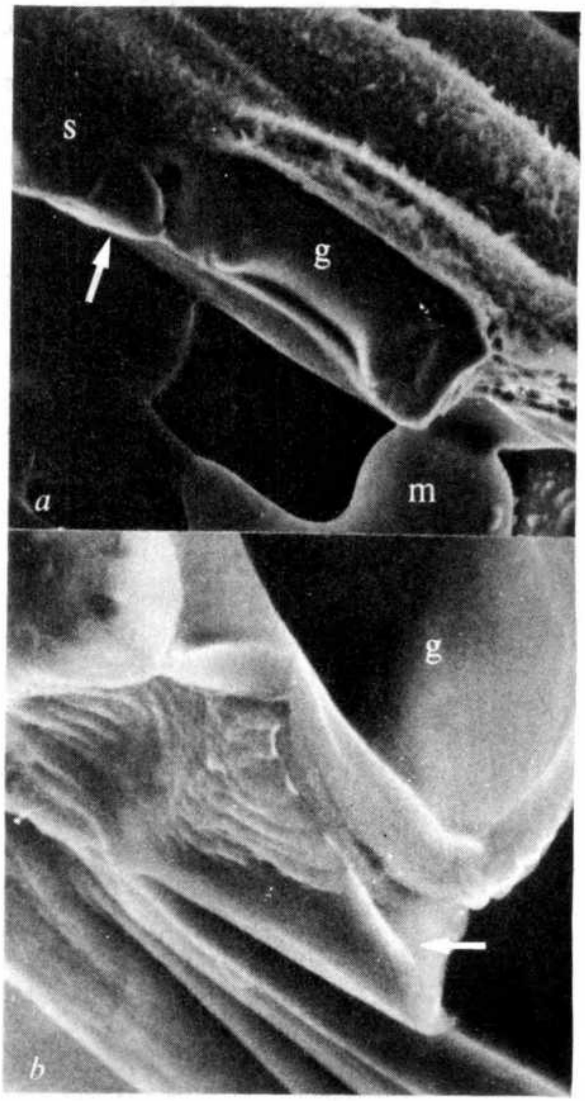


Fig. 2 *a*, Scanning electron micrograph of a vertical longitudinal section through the lower epidermis of *Tradescantia pallidus*. The section passed exactly through the centre of a stomatal complex leaving a single guard cell (g) and polar subsidiary cell (s). The mesophyll tissue (m) indicates the orientation of the section. An ion-adsorbent body (arrowed) can be seen in the intercellular space between the pole of the guard cell and the adjacent subsidiary cell (× 1,000). *b*, Scanning electron micrograph of a vertical section through the polar region of a guard cell (g) of *Tradescantia pallidus*. The internal paradermal wall of the guard cell has laminate structure with free spaces (arrowed) lying between successive layers (× 5,000).

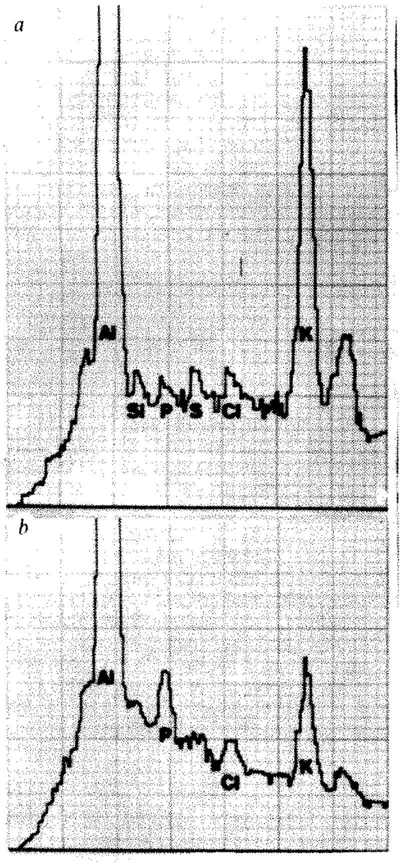


Fig. 3 Preliminary electron microprobe analyses of the stomatal complex of *Tradescantia pallidus*. The probe was about 6 µm in diameter at an accelerating voltage of 18 keV for 100 s. The peak to the immediate right of the potassium peak in the spectra is of a composite nature in that it is made up of potassium K_{β} and calcium K_{α} emissions. *a*, K_{α} spectral emission from the ion-adsorbent body illustrated in Fig. 2*a*; *b*, K_{α} spectral emission from the polar region of the adjacent guard cell illustrated in Fig. 2*a*.

with the guard cell lumen volume of about 7.85×10^{-15} m^3; the combined volumes of a pair of ion-adsorbent bodies associated with a single guard cell, is, therefore, in the order of 7% of the guard cell volume.

When viewed from the side adjacent to the mesophyll, the bodies can be seen to be covered by the endocuticle, which is strongly plicated at the pole of the guard cell and over the immediately adjacent areas of the polar subsidiary cells, the bodies are similar to the trabeculae reported on from the stomata of *Polypodium vulgare*[10]. Sections through the polar region of the guard cells indicate that the extracellular space, in which the ion-adsorbent bodies are located, is contiguous with spaces present in the matrix of the lower paradermal wall of the guard cell (Fig. 2*b*). These spaces are not apparent in thin sections observed under the transmission electron microscope (probably as a result of fixation procedures), although the differentially-thickened cell wall in this region seems to be strongly laminate.

Electron microprobe analysis of the ion-adsorbent body (Fig. 3*a*) suggests that it is particularly rich in potassium compared with the protoplast at the pole of the adjoining guard cell (Fig. 3*b*). In addition, the body also contains appreciable quantities of silicon, sulphur, phosphorus and chlorine, while only the latter two are detectable in comparable amounts in the guard cell protoplast.

Our findings conform to the hypothetical arrangement proposed for the substomatal structures as found in *Polypodium vulgare*[10]. The ion-adsorbent bodies are situated in an extracellular position in both species studied, although their exact location differs. The bodies are in close association with the outer face of the lower paradermal cell walls at the poles of the guard cell complex in *Polypodium vulgare*, while in *Tradescantia pallidus* they are situated in the intercellular space between the pole of the guard cell complex and the polar subsidiary cells.

Potassium is the most important cation involved in the stomatal mechanism and the considerable concentrations of the ion detected in the ion-adsorbent bodies must strongly implicate their involvement in stomatal movements. Being situated as they are at the end of the epidermal water pathway[12], the bodies are in a strategic position to adsorb potassium, among other ions, from the transpiration stream. The bodies can thus have a dual role. They maintain a store of bound potassium which can be released, as and when required, for incorporation into the guard cell vacuole. At the same time, they remove other potentially toxic ions from the transpiration stream (our unpublished data) which could otherwise become lodged elsewhere in the apoplast, and possibly be taken up in the symplast, as water leaves the tissues at the evaporative sites around the stomata.

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Heterogeneous population of mitochondrial DNA molecules in higher plants

THE physicochemical properties of mitochondrial (mt)-DNA of higher plants have been reported[1–4]. mt-DNAs occur as closed circles with molecular weights of $(60\text{–}70) \times 10^6$ and it is likely that each mitochondria contains several of these circles. mt-DNAs isolated from different higher plants exhibit similar physicochemical parameters: all mt-DNAs so far analysed[1–5] in higher plants band at a unique buoyant density of 1.706 g cm^{-3} in neutral CsCl gradients and the few published melting points and reassociation kinetics are not significantly different[2–4]. Not enough values have been reported to state on possible differences in the contour length measured by electron microscopy. Thus, mt-DNAs isolated from different higher plants cannot be specifically identified by conventional physicochemical techniques. We have recently reported that the analysis of restriction nuclease digests by gel electrophoresis provides a very sensitive test to identify specifically chloroplastic (cp)-DNAs isolated from different plant genera and species[6]. We show here that this technique also allows the characterisation and the specific identification of mt-DNAs isolated from various higher plants. We used *Eco*RI specific cleavage to evidence the general occurrence of heterogeneous population of mt-DNA molecules within an higher plant and to detect clear differences between mt-DNAs extracted from normal and cytoplasmic male-sterile wheat.

Etiolated seedlings of *Cucumis sativus* L. var. Vert long maraîcher, *Triticum aestivum* var. Capitole, *T. aestivum* var. Florence Aurore and *T. aestivum* var. Gaines (normal and male sterile lines) were used. *Solanum tuberosum* var. Bintje was grown as callus tissue[7]. Liquid cell suspensions of normal and

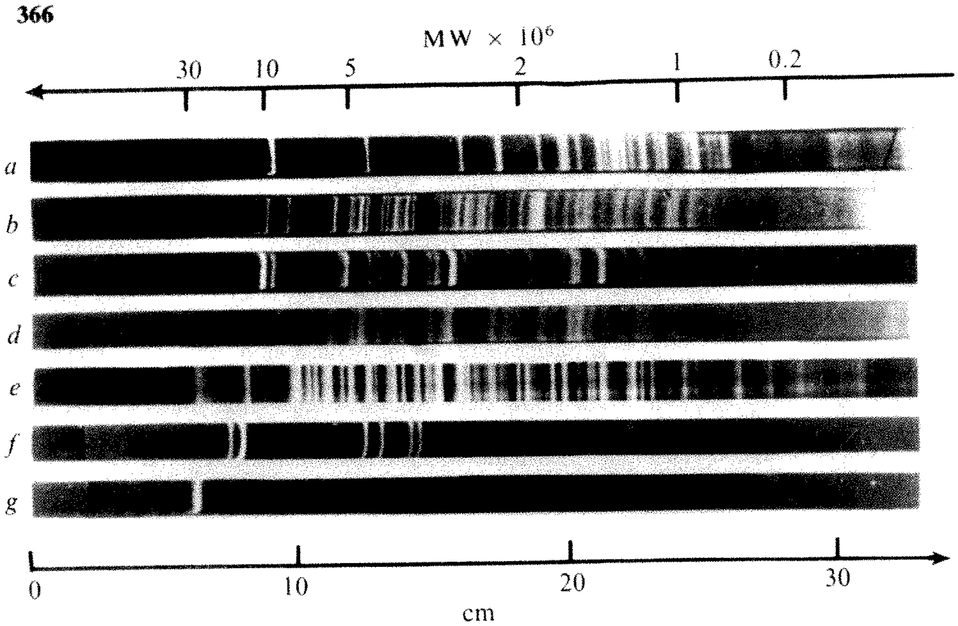

Fig. 1 Agarose slab gel electrophoresis of *Eoc*RI digests of mt-DNAs from: *b*, wheat (*T. aestivum* var. Capitole); *c*, potato; *d*, cucumber; *e*, virginia creeper. Wheat (*T. aestivum* var. Capitole) cp-DNA (*a*) and λ*plac*5 DNA (*f*) were used for comparison and molecular weight standards respectively. The arrow indicates the direction of migration. Homogenisation of plantlets (etiolated, 10-cm long, roots removed) was carried out with a Waring blender (3×5 s, maximum speed); calluses and liquid cultured cells were disrupted by a French press (3,000 p.s.i.) in buffer A (ref. 10). The homogenate was filtered through a 40-μm nylon net, centrifuged at 1,200*g* for 10 min and the supernatant spun at 15,000*g* for 15 min. The pellet was dispersed with a Potter homogeniser, recentrifuged at 1,200*g* and 15,000*g*, and dispersed again. A DNase treatment was achieved at 50 μg cm^{-3} (DNase I), 4 °C for 1 h and the DNase was removed by 2 washings in buffer B (ref. 10) at 15,000*g* for 15 min. The dispersed pellet was loaded on a 30–60% sucrose gradient in buffer B; after 1 h at 22,000 r.p.m. in an SW.25 II rotor, the mitochondrial band was recovered, diluted with buffer B and pelleted. DNA was isolated as described[6] for cp-DNA. The supercoiled DNA band was diluted with buffer C (ref. 10) and run at 35,000 r.p.m. overnight in an SW41 rotor. The pellet was suspended in 0.1 M NaCl, 10^{-2} M Tris, 10^{-3} M EDTA, *p*H 8 and the liquid was a cleared by a 30-s centrifugation at 20,000*g* (Eppendorf microcentrifuge); the drug was removed by chromatography on a Dowex 50 Na^+ column (20×4 mm). Purity and circularity were checked as for cp-DNA (ref. 6). *Eco*RI was purchased from Miles or prepared according to ref. 11. Experimental conditions for digestion, electrophoresis and ultraviolet fluorescence photography were as before[6], except that the 0.7% Agarose electrophoresis was achieved on 38-cm long, 15-cm wide (eight slots), 4-mm thick slab gels.

tumorous *Parthenocissus tricuspidata* L. were cultured in darkness[8] and collected during the exponential growth phase. Mitochondria were purified by two cycles of differential centrifugations and cleared from contaminating DNA by a DNase treatment. Mitochondria were freed from DNase by two washes and further purified by a sucrose gradient. The supercoiled mt-DNAs were isolated as the lower ultraviolet fluorescent band of propidium diiodide or ethidium bromide CsCl gradients[6] and made drug-free by a short Dowex chromatography. The purity was checked by analytical CsCl gradients (unimodal and sharp band at 1.706 g cm^{-3}) and the circularity was controlled by a Kleinschmidt spreading followed by positive staining with acid–alcoholic uranyl acetate[9]. The purified mt-DNAs were then digested by saturating amounts of *Eco*RI, *Bam*I or *Sal*I, and the digests analysed by electrophoresis on 38-cm long, 0.7% Agarose slab gels. *Eco*RI restriction fragments given by λ*plac* 5 DNA and by wheat cp-DNA were used as molecular weight standards. Gels were stained with ethidium bromide and ultraviolet fluorescence photographs were taken.

Figure 1 shows the electrophoretic pattern of the mt-DNAs extracted from cucumber, potato, wheat and virginia creeper. Some 50 distinct bands were resolved in each case, about 40 moved more slowly than the bromophenol blue. Like cp-DNA, the mt-DNA of a given plant seems to be characterised by a specific digestion pattern and may be easily distinguished from that of another plant belonging to a different genus. These patterns were found easily reproducible: several independent preparations of each mt-DNA were individually digested and lead to rigorously reproducible banding patterns, whatever the digestion time (3–12 h). The same number of bands was obtained on 58-cm Agarose gels in individual tubes.

mt-DNA isolated from three hexaploid wheats, *Triticum aestivum* var. Capitole, *T. aestivum* var. Florence Aurore and *T. aestivum* var. Gaines display an identical pattern (Fig. 2,

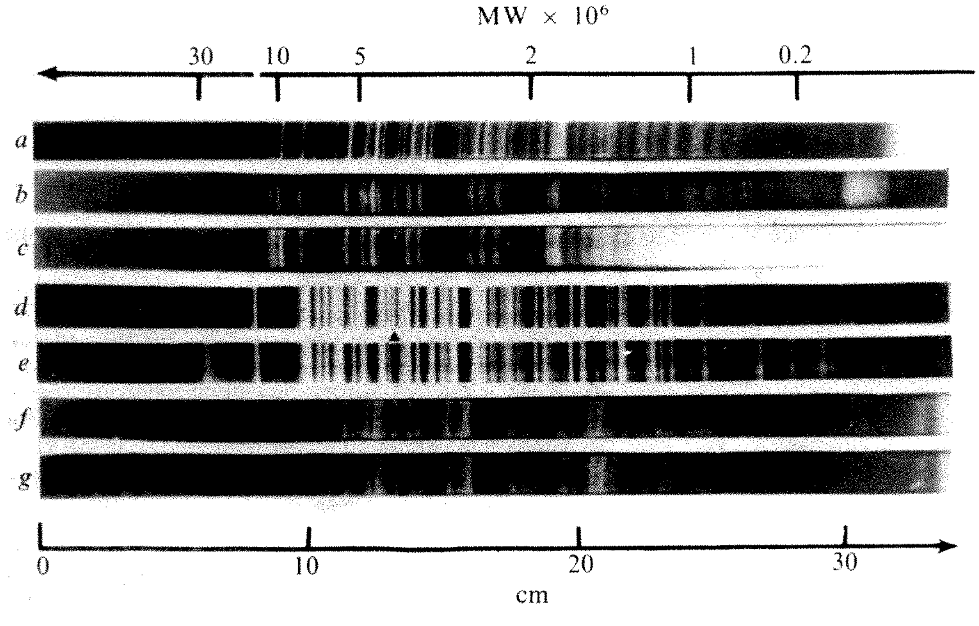

Fig 2 Agarose gel electrophoresis of *Eco*RI digests of mt-DNAs isolated from: *a*, *Triticum aestivum* var. Capitole; *b*, *T. aestivum* var. Florence Aurore; *c*, *T. aestivum* var. Gaines; *d*, liquid cultured normal cells of *Parthenocissus tricuspidata* L.; *e*, liquid cultured crown-gall cells of *P. tricuspidata* L.; *f*, cucumber hypocotyls; *g*, cucumber cotyledons. Isolation and anlaysis were performed as for Fig. 1.

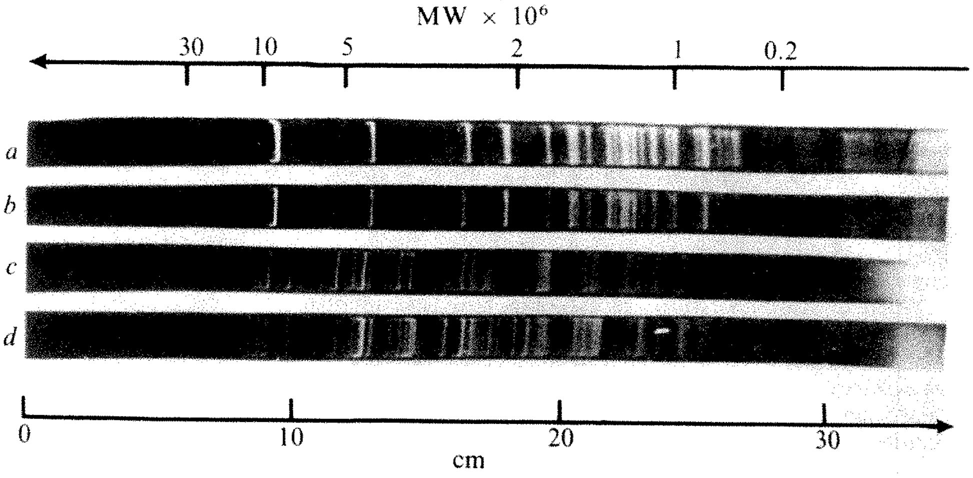

Fig. 3 Agarose gel electrophoresis of *Eco*RI digests of *a*, cp-DNA isolated from normal *T. aestivum* var. Gaines (cytoplasm *T. aestivum*); *b*, cp-DNA isolated from cytoplasmic male-sterile *T. aestivum* var. Gaines (cytoplasm *T. timophevii*); *c*, mt-DNA from normal *T. aestivum* var. Gaines; *d*, mt-DNA from cytoplasmic male-sterile *T. aestivum* var. Gaines. Isolation and analysis were performed as described in Fig. 1.

gels *a–c*); this identity is direct evidence for the isocytoplasmic origin of these varieties, deduced from interspecific reciprocal crosses[12]. The corresponding cp-DNAs also led to identical patterns (results not shown).

The sensitivity of this method is illustrated on Fig. 2, gels (*d*) and (*e*), where mt-DNAs extracted from normal and tumorous cell suspensions of virginia creeper differ only by one band among 50; whereas this difference is highly reproducible, it is not known however whether this DNA modification is related to the tumorous transformation or simply results from an *in-vitro* evolution of the strains. Gels (*f*) and (*g*) in Fig. 2 show that mt-DNAs extracted from two physiologically different organs, cotyledons and hypocotyls, of a given plant (cucumber) do not present any detectable difference.

The cytoplasmic male sterility which affects many higher plant species has been claimed to result from mutation(s) in chloroplastic and/or mitochondrial DNAs (ref. 13). The mt-DNAs have been isolated from normal *T. aestivum* var. Gaines (cytoplasm *T. aestivum*) and from cytoplasmic male sterile *T. aestivum* var. Gaines (cytoplasm *T. timophevii*). Figure 3 *c, d* shows that the male sterile mt-DNA is altered for several bands; such a difference has been recently reported for cytoplasmic male sterile maize[14]. Since we have checked here that cp-DNAs of normal and male sterile lines are identical (Fig. 3, *a, b*) the male sterility mechanism seems to only involve the mitochondria.

All these mt-DNAs led to about 50 bands when split by *Eco*RI; this number is quite larger than that obtained with cp-DNAs, as illustrated on Fig. 1 (see ref. 6). The fluorescence intensity of several bands is greater than expected from normal stoichiometry and reflects the presence of multiple fragments. Even when these band multiplicities are not taken into account, the sum of the band molecular sizes estimated for virginia creeper, cucumber and wheat mt-DNAs were respectively 165×10^6, 120×10^6 and 140×10^6; these values are much larger than the native molecular weight (60 to 70×10^6) directly determined from the contour length by electron microscopy (pea[3]; virginia creeper, our unpublished results). The sum is only 90×10^6 for potato calluses mt-DNA, probably because of the presence of a multiple band of high molecular weight (first band on Fig. 1 gel *c*). This value, 90×10^6, is nevertheless larger than the native molecular weight estimated from the contour length (60×10^6, data not shown). All these banding patterns were rigorously reproducible in respect to both the relative position and the relative intensity of the bands; several DNA isolations, different batches of enzyme, different concentrations of enzyme, extended digestion time (12 h), phenol-treated digests and digests heated to 60 °C gave identical distributions. These electrophoregrams therefore represent non-reassociated limit digests.

The mt-DNA of *T. aestivum* var. Capitole has been exhaustively digested (7 h) by two other restriction endonucleases, *Bam*I and *Sal*I. The electrophoretic patterns are shown in Fig 4 and confirm that the sum of the fragment sizes remains quite larger than the native molecular weight, even when band multiplicities are not taken into account. The possibility that such discrepancies could result from a methylation cannot be ruled out, since methylation of a base in either strand of the endonuclease recognition site is sufficient to prevent the endonuclease from attacking the sequence[15]. Whereas it is agreed that cp-DNA does not contain 5-methylcytosine[16], no information is available about the presence of methylated bases into the mt-DNA of higher plants. This methylation should not occur randomly since the electrophoretic pattern remains unaltered (in number and size of fragments and in relative intensity of band fluorescence) for a given plant species, whatever the organ, the age of the plant or the growth conditions (data not shown).

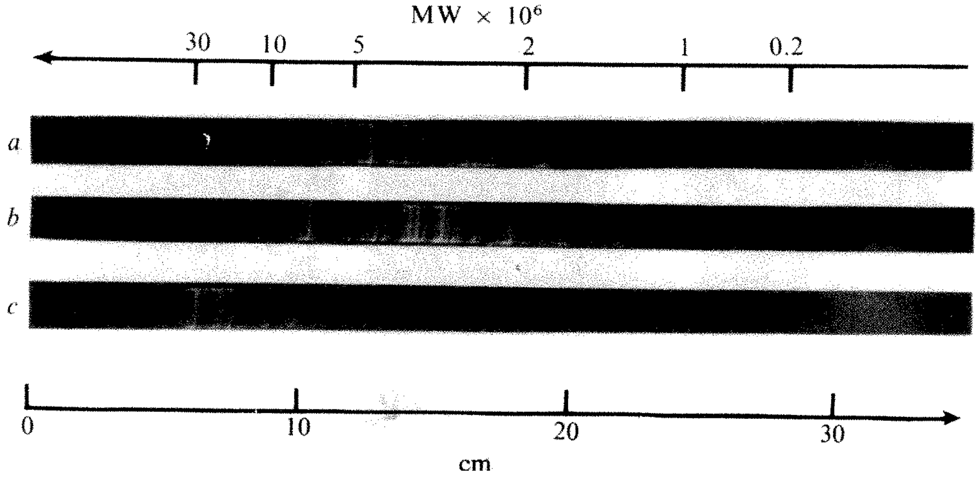

Fig. 4 Agarose gel electrophoresis of *Eco*RI (*a*), *Bam*I (*b*), and *Sal*I (*c*) digests of mt-DNA isolated from *T. aestivum* var. Capitole. Experimental procedure as for Fig. 1.

If methylation is to be ruled out, the main conclusion arising from above molecular weight discrepancies should be that the population of mt-DNA molecules is heterogeneous and that an higher plant contains different mt-DNA molecules. The plant mitochondrial genome would be constituted by several circular mt-DNA molecules with identical buoyant density but with some differences in sequence arrangement. The mitochondrial genome complement would be represented either by one mitochondria containing different circular mt-DNA molecules or by several classes of mitochondria[17], each containing one type of DNA molecule.

Whereas inter- and intraspecies variations have been detected for mammalian mt-DNAs, the mt-DNA of each studied mammal seems to be constituted by a single molecular clone, as evidenced by the good correspondence between the contour length and the molecular weight estimated from *Hae*III restriction patterns[18]. Higher plant mitochondria thus differ from mammalian mitochondria in this respect. Heterogeneity of this type has been reported for the kinetoplast DNA isolated from *Crithidia luciliae* and split by *Hap*II restriction nuclease[19].

The determination of mt-DNA restriction patterns, together with study of the chloroplastic DNA (ref. 6), should be very useful in analysis of phylogenies and in determining the nature of parasexual hybrids.

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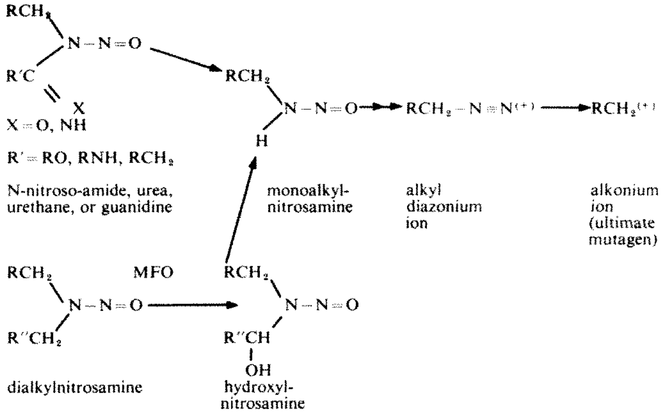
Inhibition by L-ascorbate of bacterial mutagenesis induced by two *N*-nitroso compounds

MANY chemicals are activated to carcinogens and mutagens by oxidative metabolism to alkylating agents[1]. Ascorbic acid has been reported to protect against tumour induction in mice by 3-hydroxyanthranilic acid, which induced bladder tumours[2] and 7,12-dimethylbenzanthracene plus croton oil which induced skin tumours[3]. Ascorbic acid can also prevent the formation of carcinogenic *N*-nitroso compounds from nitrite and amines, *in vivo*[4] and *in vitro*[5]. I report here that mutagenesis by two preformed *N*-nitroso compounds is effectively inhibited by ascorbic acid.

Many *N*-nitroso compounds are potent carcinogens and mutagens[6–8]. The mechanism of action of these compounds is believed to involve decomposition to short-lived highly reactive electrophiles which react with nucleophilic sites on DNA bases leading to altered bases[6–8]. Some of these alterations may not be fully repairable in certain tissues, and cause base pair substitution mutations, and perhaps lead to tumour formation[9,10]. There is evidence that the reactive electrophile and ultimate mutagen is an alkonium ion, which is formed by rapid intramolecular rearrangements subsequent to spontaneous scission of the derivatised α carbon of *N*-nitroso-amides, ureas, urethanes or guanidines[6–8]. *N*-nitrosamines also decompose to yield alkonium ions, but as their *N*-α-carbon bonds are not labile, nitrosamines must be metabolically activated by an αhydroxylase activity present in the mixed function oxidase (MFO) system[6–8].

These reaction pathways are depicted below:



As both types of *N*-nitroso compounds are believed to generate altered bases by an electrophilic attack on nucleophilic sites in DNA, it seems possible that an exogenous nucleophile could compete with DNA bases for the ultimate mutagen and thus serve as a possible preventative agent against mutagenesis and carcinogenesis. As tissue and biological fluids contain nucleophilic compounds which could also react with alkonium ions, this nucleophile would have to be more reactive than these endogenous nucleophiles if it were to be effetive. Alternatively, it could be of comparable nucleophilicity but be present in higher concentrations than endogenous nucleophiles in the proximity of DNA. It should also be non-toxic at the levels used. One such compound might be L-ascorbic acid. Although usually known for its anti-oxidant properties, L-ascorbate which is the predominant form at physiological *p*H, possesses substantial nucleophilic character[11]. In fact, it has been suggested by Edgar that ascorbate might protect against electrophilic attack on cellular DNA by intercepting alkylating agents[11]. In addition, it can be ingested in moderate quantities without apparent toxicity[12,13]. In this study the effects of ascorbate ion on microbial mutagenesis by *N*-methyl-*N*-nitrosoguanidine (MNNG) and dimethylnitrosamine (DMN), were examined.

Mutagenesis was assayed by a bacterial auxotroph reversion test using the histidine requiring *Salmonella typhimurium* tester strain TA 1530 described by Ames *et al.*[14]. The microorganisms were incubated with MNNG or DMN in 0.1 M phosphate buffer, *p*H 7.4, in the presence or absence of ascorbic acid. When DMN was used, a rat liver extract, containing the MFO system was incorporated into the incubation mix[15]. Aliquots were taken at several incubation times and reversion of microorganisms to histidine independence was assayed as described in Fig. 1.

In Fig. 1 the inhibitory effects on MNNG mediated mutagenesis by various concentrations of ascorbic acid are shown. Higher concentrations of ascorbate caused greater inhibition of mutagenesis. Mutagenesis by DMN, (Fig. 1*c*, *d*), is also inhibited by ascorbate. Higher concentrations of ascorbate are necessary to inhibit mutagenesis by DMN than by MNNG. The concentration of ascorbate necessary to produce a particular percentage inhibition of DMN-induced mutagenesis is a function of the concentration of liver extract in the incubation mixture. For example, when DMN was activated by

1.5 mg ml^{-1} liver extract, 90% inhibition was afforded by 1.5 mg ml^{-1} ascorbate. When twice the concentration of liver extract was used and other conditions held constant about twice the concentration of ascorbate was required for the same percentage inhibition. As both DMN and MNNG are believed to yield the same ultimate mutagen[6], it seems that the components of the liver extract rendered ascorbate a less effective inhibitor, since the MNNG mutagenesis system contained no added activation system. Since proteins contain a number of side chains with nucleophilic moieties it is possible that some of these may compete with ascorbate by reacting with the ultimate mutagen, thus decreasing the life-time of the mutagen. This effect seems to be important since added bovine serum albumin, 2 mg ml^{-1} in the absence of ascorbate, inhibited mutagenesis by MNNG by over 90%.

Alternatively the liver extracts might bind or deactivate ascorbate and thus lower its effective concentration. Some indirect evidence of this latter effect may be seen in the fact that in the absence of liver extracts ascorbic acid is toxic to the bacteria at concentrations above 0.3 mg ml^{-1}, but in the presence of liver extracts no toxicity is observed at concentrations as high as 3.0 mg ml^{-1}. Ascorbate toxicity has been reported with other strains of bacteria[16]. No significant toxicity was observed in the conditions used in this study. In view of these complications and the possibility of ascorbate interference in the activation of DMN to a mutagen, further studies were carried out with simpler MNNG system.

If there is in fact competition between ascorbate and microorganisms for the ultimate mutagen, then a further demonstration of this competition would be a decreased efficiency of inhibition by ascorbate at higher concentrations of microorganisms. As shown in Table 1, the percentage inhibition by ascorbate drops as the concentration of bacteria in the incubation mix increases. This effect and the increasing inhibition at higher ascorbate concentrations described above suggest that ascorbate exerts its inhibitory effect by trapping the ultimate mutagen before it interacts with cellular DNA.

Other biological nucleophiles also inhibited mutagenesis by MNNG (see Table 2). But none of those tested was as effective as ascorbate. The compounds tested were chosen because they contain most of the functional groups that are found in significant concentrations in biological fluids[17]. The actual concentrations of these compounds relative to ascorbate will vary in different tissues and with ascorbate intake. In urine for instance, ascorbate concentrations may be considerably higher than those used in this study[18], whereas the concentrations of the compounds in Table 2 are usually significantly lower[17].

The concentration of ascorbate necessary to effectively inhibit mutagenesis by MNNG can readily be exceeded in the urine[18], and may be reached in certain other tissues[18]. As this study indicates, a number of compounds normally found in urine are capable of inhibiting mutagenesis by MNNG; however, ascorbate is the most effective. It cannot be excluded, however, that ascorbate might be able to deactivate the parent *N*-nitroso compounds by some other mechanism such as reduction, which is not available to most of the compounds listed in Table 2. Several *N*-nitroso compounds are known bladder carcinogens in animals[8]. If the mechanisms of carcinogenesis and mutagenesis are similar[19], and if *N*-nitroso compounds are bladder carcinogens in humans, then some protective effect against these compounds might be afforded by saturating doses of vitamin C. In previous studies where ascorbate inhibited carcinogenesis its protective role was attributed to its role in preventing oxidative activation of carcinogens[2,3], presumably to electrophiles. It is possible that ascorbate may also exert a protective effect by intercepting electrophilic

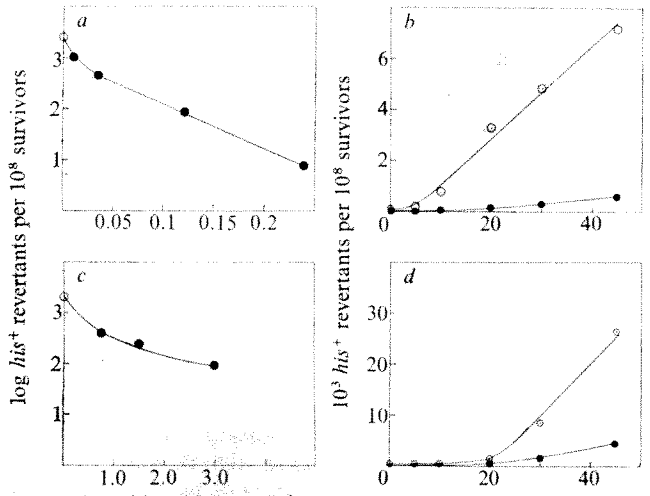


Fig. 1 Effect of ascorbate on mutagenesis of *Salmonella typhimurium* TA 1530 by MNNG and DMN. *a*, *c*, Effect of concentration of ascorbate on mutagenesis by MNNG and DMN respectively; *b*, *d*, effect of incubation time on mutagenesis by MNNG and DMN respectively. Reaction mixtures (0.5 ml) contained: bacteria, 2 × 10^8 c.f.u. centrifuged from a stationary phase culture; MNNG, 8.0 μM (*a*), 13 μM (*b*) or DMN 200 mM + liver extract from Aroclor pretreated rats[15], containing 1.5 mg ml^{-1} protein (*c*) or 3.0 mg ml^{-1} (*d*); 0.1 M phosphate buffer (*p*H 7.4); ascorbic acid, 0.12 mg ml^{-1} (*b*), 3.0 mg ml^{-1} (*d*) or varying concentrations (*a*) and (*c*). ○, no ascorbate; ●, ascorbate. Test tubes containing the incubation mix were shaken aerobically at 37 °C, for 15 min (*a*), 40 min (*c*) or varying times, (*b*) and (*d*). Bacteria were assayed for histidine independent (*his*$^+$) revertants and cell viability. Cell viability was not significantly decreased during the incubation. The spontaneous revertant frequency was < 10 per 10^8 c.f.u. and was not subtracted from the total revertants. Ascorbate may have been slightly mutagenic, as in the absence of added mutagen it caused a doubling in the revertant frequency. Revertant frequencies obtained from duplicate assays were within 15% of each other. *S. typhimurium* TA 1530 was kindly provided by B. Ames.

Table 1 Effect of concentration of bacteria on inhibition of MNNG mediated mutagenesis by ascorbate

Bacteria (× 10^8 c.f.u. per m)	Revertants ml^{-1} No ascorbate (R_0)	Revertants ml^{-1} Ascorbate (R_a)	R_a/R_0
2.6	3,380	300	0.088
4.6	4,840	1,260	0.26
6.5	11,010	3,590	0.33
13.0	14,020	6,250	0.45

Mutagenesis was assayed as described in Fig. 1. The incubation time was 15 min, the concentration of MNNG was 8.0 μM and that of ascorbic acid 0.06 mg ml^{-1}. c.f.u., Colony forming units.

Table 2 Effects of various biological compounds on mutagenesis by MNNG

Addition (mM)	$\frac{10^3 \text{ Revertants}}{10^8 \text{ c.f.u.}}$
—	3.0
Ascorbic acid, 6.8	0.04
Creatine·H_2O, 8.1	0.69
Cysteine·HCl, 7.6*	3.3
Methionine, 8.1	2.5
Serine, 11.4	0.43
Tyrosine, 6.6	0.62
Trypotphan, 5.9	0.54

Mutagenesis assay was as described in Fig. 1. The incubation time was 8 min and the concentration of MNNG was 8.0 μM. All additions were present at a final concentration of 0.12 mg ml^{-1}. Similar percentage inhibition was seen at longer incubation times.

*Cysteine became inhibitory at longer incubation times. At 2 h incubation when mutagenesis had nearly ceased it had inhibited mutagenesis by 50%. It was the only compound in this table to show this effect.

metabolites, or their precursors, independent of their mode of production.

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Note added in proof: Recent experiments have shown that addition of ascorbate to solutions of MNNG causes a rapid decrease in the absorbance of MNNG at 400 nm, whereas addition of the same concentrations of the other compounds in Table 2 had little effect. Some of the MNNG may thus be directly deactivated by ascorbate.

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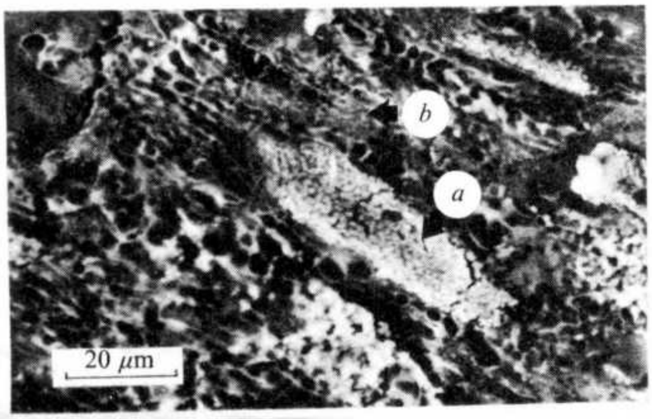
Intracellular cationic counterion composition of an acid mucopolysaccharide

EVEN though much is known about biopolymer synthesis and secretion, little or nothing is known about the exact identity of counterions bound to charged biopolymers inside cells. This may be very important since bound ions can influence polymer size and shape and so may hold answers to questions about polymer packaging in cells. In this paper we demonstrate how X-ray microprobe analysis of frozen mucus cells, from a marine snail, has enabled us to show that a sulphated mucopolysaccharide only binds potassium inside the cell but releases this to bind magnesium and calcium after secretion. This is of particular interest because, of all the common physiological cations, only potassium precipitates this mucopolysaccharide *in vitro*. The implication is that potassium could cause the polymer to adopt a more compact form within the cell.

Fig. 1 Scanning electron micrograph of a freeze-fractured face in the hypobranchial gland epithelium of the gastropod *Buccinum undatum*. Arrow A points to an acid mucopolysaccharide cell filled with vesicles while arrow B indicates an area of ciliated and interstitial cells. Both areas A and B were probed for elemental analysis by X-ray emission. The results are shown in Fig. 2 and Table 1. Probe spot diameters of about 2 µm were used.



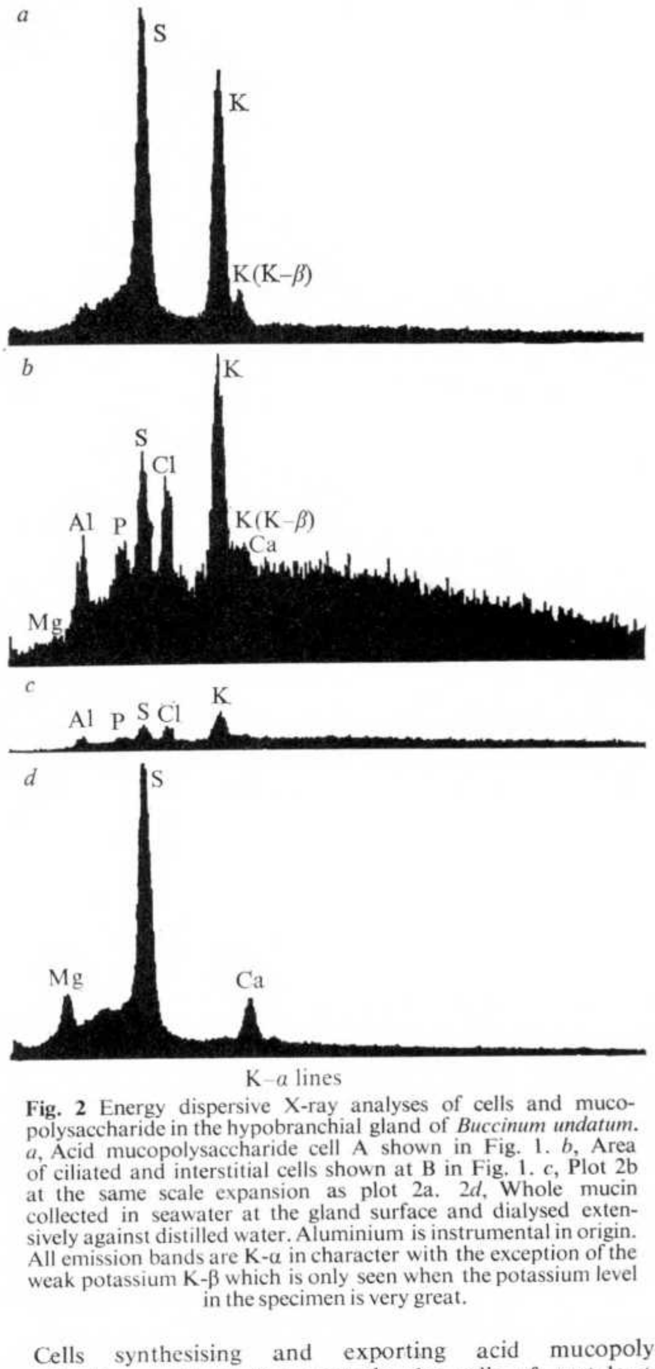


Fig. 2 Energy dispersive X-ray analyses of cells and mucopolysaccharide in the hypobranchial gland of *Buccinum undatum*. *a*, Acid mucopolysaccharide cell A shown in Fig. 1. *b*, Area of ciliated and interstitial cells shown at B in Fig. 1. *c*, Plot 2b at the same scale expansion as plot 2a. 2*d*, Whole mucin collected in seawater at the gland surface and dialysed extensively against distilled water. Aluminium is instrumental in origin. All emission bands are K-α in character with the exception of the weak potassium K-β which is only seen when the potassium level in the specimen is very great.

Cells synthesising and exporting acid mucopolysaccharides, such as for example the cells of vertebrate cartilage or the epithelial gland cells of many marine invertebrates, must experience a problem of cationic balance. From the moment that the polymer first receives its charged groups, whether these be carboxyl or sulphate, the intense electrostatic field generated by the polyanion will attract an atmosphere in which cations will predominate; moreover, as with polyelectrolytes generally, a substantial proportion of these cations will be bound specifically as ion pairs. There is evidence indicating that there may be orders of preference of binding different cations by differing anionic groups[1,2] and suggesting that different polymers may show different cation specificities[2]. The type of counterion bound may in some cases radically affect the polyanion conformation[3].

Whether polyanion synthesis is accompanied by a continuous export from the cell or by storage in vacuoles for bulk secretion, the cytoplasm of the cells would experience an unusual demand on the free cation pool, which has to be restored by inward transport of cations. Moreover, should the polyanion show cation specificity, unusual imbalance in the cation composition of the cell may develop with obvious implications. These are possibilities which can only be speculated on in the absence of information about the counterion distribution surrounding polyanions.

Although determination of the ion-binding properties of an acid mucopolysaccharide outside the cell is relatively easy, it has hitherto been impossible to determine the intracellular counterion distribution accompanying the polyanion. We have now carried out such an analytical electron microscopic study using a secretory epithelium from the marine gastropod *Buccinum undatum*. We chose this tissue rather than vertebrate connective tissue cells for an initial investigation because the polysaccharide produced is fairly simple, containing only ester sulphate and no carboxyl groups[4,5], the tissue has been well described ultrastructurally[6], the secretion vesicles inside the cell are readily seen in the electron microscope[6] and finally, because the presence of ester sulphate and little or no amino acid sulphur in the mucopolysaccharide allows for straight comparison between sulphur and cation X-ray signals in the analytical microscope. We used the hypobranchial gland, which secretes a mucin into the mantle cavity of the mollusc and is a folded epithelium with a large population of secretory and ciliated cells. Mucin forms by the mixing of two secretion products at the gland surface. One product, the polyanion, is essentially cellulose with one ester sulphate residue per glucose unit[4,5], and the other, formed in a different cell type[6], is a neutral glycoprotein.

Since any form of fixation, dehydration and embedding would have radical effects on cation content in the tissue we adopted the strategy of quench–freezing freshly excised tissue in 'slushy' liquid nitrogen, placing the frozen specimen in the scanning electron microscope and cleaving the tissue on the freezing stage *in vacuo* with a scalpel blade. The cells are exposed in fractured section and vacuum sublimation sufficiently etches the cleft surface to make tissue topography readily recognisable when compared with conventional micrographs[6]. Figure 1 shows such a cleft surface, acid mucopolysaccharide cells are clearly distinguishable from other cells. Electron beam probing and energy dispersive X-ray analysis applied to the secretion vacuoles of these cells gave the results typified in Fig. 2*a*. Interestingly high sulphur and potassium peaks dominated the spectrum and no other cation peaks were seen in these plots. Probing non-mucopolysaccharide cells in the tissue gave results typified by Fig. 2*b* and *c*. These are shown at the same and greater scale expansion as the acid mucopolysaccharide cells, to emphasise the high levels of potassium and sulphur present in the secretory vaccuoles relative to the ionic content of the general cytoplasm of other cells.

Since we had shown that the polyanion probably exists in the cell solely as the potassium salt, we tried to determine whether the counterion is altered when the polyanion is released into the seawater medium. It is at this time also that polyanion and glycoprotein interact to form a rheotropic mucin. Figure 2*d* shows the X-ray analysis of a mucin sample collected under seawater as it left the gland. The mucin had been transferred immediately to distilled water, dialysed extensively and freeze-dried. Potassium has gone from the trace completely and has been replaced by magnesium and calcium. Sodium too, is absent. Dialysis of the mucin against seawater before distilled water does not alter this pattern.

A difficulty about this technique is its semi-quantitative nature. X-ray counting efficiency falls off rapidly for atomic numbers below 15 and so on an atom-to-atom basis sulphur and sodium peaks should be in approximately 10 : 1 ratio, whereas equivalent heights of sulphur and potassium peaks are more nearly indicative of equivalent atomic ratios. Nevertheless, the height of the magnesium peak indicates that sodium would be detected if present.

In Table 1 we show the integrated values of the elemental peaks seen in Fig. 2*a*–*c* as well as values for scans of the purified acid mucopolysaccharide as its potassium and sodium salts and in the ionic form generated by dialysis of the potassium salt against seawater. Attempts to replace potassium by sodium using high salt concentrations or sodium-form ion-exchange resins fail to remove all potassium. The reverse is not true. Like whole mucin, pure polyanion exposed to seawater loses all detectable monovalent cation, gaining mainly magnesium and somewhat less calcium.

Thus it seems that the polyanion is synthesised and stored as the potassium salt but changes in the sea to the magnesium–calcium form, in spite of the availability of sodium, magnesium and calcium inside the cell. Intracellular ionic compositions are difficult to obtain, depending on knowledge of extracellular space volumes. As an example, however, we quote analyses[7] of squid muscle cells with values of 30.8, 189, 1.88 and 19.0 mg ions per kg water for Na, K, Ca, Mg respectively, with seawater containing 492, 10.5, 10.8 and 56.1 mg ions per kg respectively of the same ions. The high concentration of sodium in seawater seems not to affect the ability of the polyanion to bind divalent ions which also compete very successfully with potassium. Since magnesium and calcium, albeit at low concentrations, are probably present in the cell why do they not also compete there with potassium for binding sites? Of interest here is the fact that concentrations of potassium ions in excess of 0.2 M *in vitro* precipitate the polysaccharide sulphate and this we use as a purification step[4]. This property is shared by some other polysaccharide sulphates, for example, K-carregeenan[8]. Excess concentrations of sodium, magnesium or calcium salts do not have this effect.

Packaging is important for the cell since most acid mucopolysaccharides hydrate readily and attempt to occupy large

Table 1 Integrated elemental X-ray counts for tissue and isolated acid mucopolysaccharide preparations from the hypobranchial gland of *Buccinum undatum*

	Na	Mg	P	S	Cl	K	Ca	Scale expansion
Acid mucopolysaccharide cell (Fig. 1)	0	0	0	26,564	0	20,559	0	4
Non-secretory cell (Fig. 1)	not measurable	120	300	1,259	995	2,122	210	7
Whole mucin after secretion into seawater	0	5,630	0	49,538	0	0	8,000	3
Acid mucopolysaccharide dialysed against seawater	0	4,669	0	63,724	0	0	7,108	3
Acid mucopolysaccharide, potassium form	0	0	0	49,524	0	38,784	0	3
Acid mucopolysaccharide, sodium form	13,860	0	0	130,728	0	6,602	0	2

X-ray energy dispersive spectra were obtained using a Jeol JSM 50A scanning electron microscope in conjunction with a Kevex analyser. Specimens were mounted on carbon stubs and maintained in the microscope at a stage temperature of −104 °C. Exciting voltages, count times and take off angles were held constant at values of 20 KV, 100 s and 30° respectively for all specimens. Vertical scale expansions were varied to accommodate X-ray yields (which are sensitive to specimen thickness and density) at major elemental peak heights. In the case of non-secretory cells X-ray continuum made sodium counting of the low levels present non-productive.

aqueous domains. These are manifested as extensive bulk-swelling properties and abnormal viscosity–concentration relationships. This is what happens here because the polymer leaves the cell and enters the aqueous medium when the released mucopolysaccharide vesicles can actually be seen in micrographs swelling and bursting and when viscosity measurements on newly released mucin show large increases in reduced viscosity accompanying dilution[5,6]. But why does the polymer remain relatively unswollen within the cell? The environment is still aqueous and a rapid and catastrophic domain expansion might be expected, but this patently does not occur. The finding that the polyanion exists intracellularly as the potassium salt, coupled with the observation that this salt is somehow different in conformation or charge distribution from other cation forms, may explain the ability of the cell to keep the polyanion packaged. This (may be achieved by maintaining specific inward membrane-mediated pumping of potassium while specific exclusion of other cations at the polyanion vesicle membranes may explain the failure of the magnesium–calcium salt to form.

We thank Professor Charles Phelps and Drs Ian Nieduszynski and John Sheenhan for helpful discussions.

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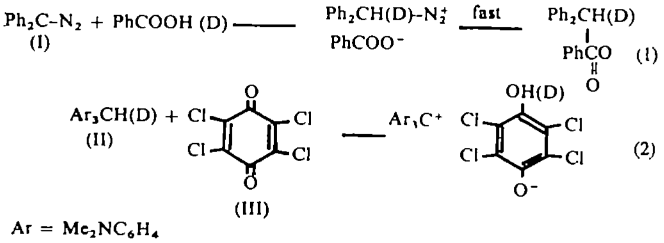
Pressure effects on proton tunnelling

We report here the first measurement of the effect of pressure on primary kinetic isotope effects of reactions in the liquid phase. We have determined rates of two reactions in solution by a sampling technique at pressures up to 2 kbar using both normal and deuterium-labelled reagents as indicated. Reaction (1) is between benzoic acid and diphenyldiazomethane (I) which has been shown to proceed by a rate-determining proton transfer[1,2]. Reaction (2) is between leuco crystal violet (II) and chloranil (III) in which the slow step is a hydride transfer[3].



In the butyl ether solvent used, reaction (1) exhibits an isotope effect which is in the normal region ($k_H/k_D = 4.5$), that is, accountable in terms of differences in zero-point energy and vibrational frequencies of reagents and transition state[4]. The abnormally large isotope effect ($k_H/k_D = 11.2$) in reaction (2) has been assumed to be due to a tunnelling contribution.

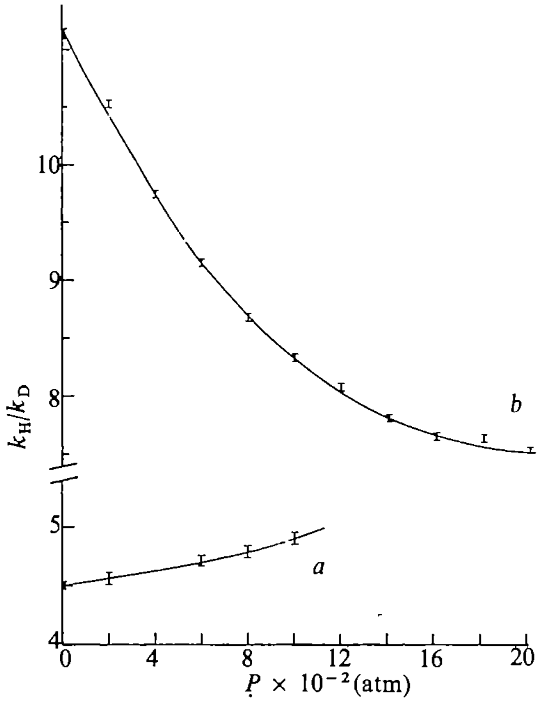


Fig. 1 Effect of pressure on the kinetic isotope effect of hydrogen transfer reactions. *a*, Reaction of diphenyldiazomethane in dibutyl ether at 26.5 °C; *b*, reaction of leuco crystal violet in acetonitrile at 29.5 °C.

We find that the behaviour of the two isotope effects under pressure is quite different. That of reaction (1) remains almost invariant while that of (2) smoothly decreases until at 2 kbar it levels off at a value near the maximum 'normal' ratio of about 7.5 (Fig. 1). It follows that the volumes of activation, $\Delta V^\ddagger$, calculated (using the regression equation $\ln k = A - \Delta V^\ddagger/RT + CP^2$)[5] for reactions (1) (H) and (1) (D) are the same (-12.8 cm^3 mol^{-1}) while those of reactions (2) (H) and (2) (D) are very divergent, being -25.5 and -35.8 cm^3 mol^{-1} respectively.

It is assumed with good justification that isotopic substitution does not alter the reaction pathway or position of the transition state along the reaction coordinate[6]; therefore it seems likely that the value of $\Delta V^\ddagger$ for reaction (2) (H) is anomalous because of a tunnelling component. In this reaction external pressure serves to lower the potential barrier to the activation process. Consequently a higher proportion of reagents will be capable of surmounting the barrier normally and the relative importance of tunnelling will diminish with increasing pressure analogous to the effect of an increase in temperature[7]. This analysis suggests that the tunnelling contribution of a reaction with a positive volume of activation (that is, which has a rate decreasing with pressure) and consequently the isotope effect, should increase with pressure. In general, where tunnelling is important, $\Delta V^\ddagger(\text{H}) < \Delta V^\ddagger(\text{D})$.

Although more data are needed to give confidence to this interpretation the result suggests that pressure dependence of the isotope effect may serve as a further criterion of the occurrence of tunnelling in hydrogen-transfer reactions.

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matters arising

Generalisation of self-thinning of plant populations

WESTOBY'S attempt[1] to generalise self-thinning of plant populations by relating leaf area per plant (*L*) to surviving plant density (*p*) is spurious. The result, therefore, is not of 'more general application than the original statement of the rule'[2].

The measured value of leaf area index (LAI) in many plant stands may vary from <1 to 20, but rarely exceeds 10 (refs 3, 4, 5). Westoby's Fig. 3 shows not merely an error in graphical labelling (the ordinate values are 10 times too large), but reveals a more fundamental misconception about the dynamics of thinning. The relationships between leaf area (*L*) and dry weight (*W*) shown in Fig. 2 are not applicable to the experimental circumstances depicted in Fig. 1 on at least two grounds. Firstly, Blackman & Wilson[6] derived their leaf area ratios (LAR= *L*/*W*) from spaced plants (~20 plants m^{-2}). LAR varies significantly with density[3,7,8] and with light intensity[6] (as Fig. 2 shows) and may vary with age[9]. Secondly, the range of *W* in Fig. 2 seldom exceeds 1.0 g, whereas its range in Fig. 1 is generally an order of magnitude greater: the extrapolation of equations derived from Fig. 2 over this range is unjustified. The neglect of density effects on LAR, coupled with graphical extrapolation cause the error in Fig. 3.

The behaviour of LAI in plant stands (both of herbs and trees) is simply stated: it rises rapidly, as a function of density and light intensity *inter alia*, to a plateau value, which is commonly <10. Thinning may occur while LAI is increasing and/or for a prolonged period while LAI remains constant, or even declines. The most general statement of the dynamics is $L = K^{-1}$ [9], where *K* varies significantly with light intensity. This is shown very clearly for *Helianthus annuus* in the original source from which Fig 1 was derived: 'the convergent leaf area index at 100%, 60% and 23% daylight was respectively 8, 6.5 and 1.5' (ref. 3), an empirical result which belies Fig. 3, where stands with least light have highest LAI for a given chronological age!

It should also be noted that the author's use of *K* in both equations relating *W* and *L* to *p* is misleading, as it implies similar constants: they are quite different.

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WESTOBY REPLIES—I thank Dr White for his helpful remarks. Firstly, he is quite correct that the scaling of the *y* axis in my Fig. 3 was ×10 too high; I apologise for confusing readers. Figure 3 was not intended as a simulacrum of a real experiment, but as a demonstration that the response of LAR to shading is the right kind to return the slope of a low-light thinning line to −3/2. That remains my basic point. Certainly LAR responds to planting density, among other factors. But does high planting density obliterate the response of LAR to shading?

I should like to suggest that perhaps the underlying determinant of relative mortality rate is how fast the light climate is deteriorating for the smallest plants in the population. Which descriptor of the population would correlate best with this process would depend on circumstances. During early growth biomass would be adequate unless LAR was varied by light treatments, in which case leaf area would be better As the death rate of leaves rises LAI asymptotes, but the location of the canopy continues to rise, which has the effect of putting the smallest plants lower in the light profile. Here cumulative production of leaf area or biomass (as measured by Hiroi and Monsi) would be the most relevant descriptor. Where stem production is cumulative and comes to dominate the biomass (for example, in tree stands), stem weight or volume will be the best available indicator of cumulative leaf area production.

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Atlantic palaeotemperatures during the Cainozoic

IT is comparatively easy to make generalisations in palaeo-oceanography, and Cifelli[1] has made several postulates regarding the Cainozoic palaeotemperatures of the North Atlantic; but the test of speculation in palaeo-oceanography is detailed biostratigraphy. For example, Cifelli[1] postulated that during the Miocene, the North Atlantic Gyre "extended perhaps 10° further north than they do now". The limited evidence, by Berggren[2], DSDP Leg 12, Sites 116 and 117 hardly support this: Berggren's[2] zonation of the Miocene planktonic foraminifera is reminiscent of that established in the cooler higher latitudes of New Zealand[3,4] than of the tropical belt zonations by Bolli[5] and Blow[6]. Work recently completed[7] on a cored Lower Miocene Burdigalian borehole sequence 110 km south-west of the Isles of Scilly has yielded an excellent planktonic foraminiferal fauna which lacks the following warm-water taxa recorded in Trinidad[2]: *Globigerina venezuelana* Hedberg, *Catapsydrax unicavus* Bolli, *C. dissimilis* (Cushman and Bermudez), *C. stainforthi* Bolli, *Globorotaloides suteri* Bolli, and *Globigerinatella insueta* Cushman and Stainforth. Conversely, the fauna contains cooler water modern species such as *Globigerina bulloides* d'Orbigny and *Turborotalita quinqueloba* (Natland) as well as cooler water extinct fossils such as *Globigerina woodi* Jenkins and *Globorotalia zealandica* (Hornibrook).

Thus from the limited, but accumulating evidence available, the planktonic foraminfera in Miocene of the North Atlantic indicate that near-surface palaeotemperatures of the ocean were not as warm as postulated by Cifelli[1].

There is well documented evidence[8] which clearly indicates that a number of species such as *Globigerinatella insueta* Cushman and Stainforth, *Globorotalia fohsi fohsi* Cushman and Ellisor, *G. fohsi lobata* Bermudez and *G. fohsi robusta* Bolli were restricted to the tropical belt in the Miocene, with cooler water in the higher latitudes such as the North Atlantic.

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CIFELLI REPLIES—Clearly, the proposal that warm gyral water was greatly expanded in the North Atlantic during the Miocene needs further testing against the evidence of the fossil record. But in dismissing this generalisation as idle speculation, Jenkins seems to have lost sight of my arguments and has shown nothing incompatible with 'detailed biostratigraphy'. DSDP Sites 116, 117 were interpreted oceanographically as gyral margin, not tropics and there is no reason to expect an identity in composition of faunal zones. Plankton assemblages of North Atlantic gyral margins are characterised by mixed associations of cool high latitude and sub tropical species plus lesser numbers of tropical expatriates displaced to the north by the clockwise movement of the circulation[1]. Mixed associations of this kind are well represented in DSDP Sites 116, 117, as discussed in my paper. If zonation of DSDP Sites 116, 117 reminds Jenkins of New Zealand zonation, then the oceanographic significance of the latter deserves reconsideration. That is, if faunal associations are mixed, as in DSDP Sites 116, 117 margins of warm gyral water may also be represented in the New Zealand Miocene.

Jenkins points out that a bore hole of Lower Miocene age near the Isles of Scilly lacks some 'warm water' species found in Trinidad. The species, however, that he lists are all extinct ones and the nature of the habitat they occupied is unknown. Actually, those species are either the same as, or closely related to, Oligocene species which are more analogous morphotypically to modern cold water than warm water forms[2]. They are a bad choice for warm water indicators.

Jenkins' claim that certain species, notably the *Globorotalia* fohsi group is restricted to the tropical belt is nullified by Bartlett[3] who recorded that species group and other tropical or subtropical forms from Nova Scotia.

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U-shaped gene frequency distributions

GILPIN *et al.*[1] have recently questioned the usefulness of the frequency distribution of allele frequencies as a means of distinguishing between the neoclassical (neutralist) and balance (selectionist) interpretations of genic variation in natural populations. The conventional view has been that random genetic drift will generate a U-shaped distribution of allele frequencies at equilibrium, while all allele frequencies will be equally probable if they represent stable equilibria maintained by the superiority of heterozygotes. Gilpin *et al.* argue that the assumption of equiprobability is gratuitous, and that the frequency distribution of allele frequencies amongst overdominant loci will be determined by the joint probability distribution of the selection coefficients acting on the homozygotes. In particular, U-shaped frequency distributions are to be expected if the probability of obtaining a given selection coefficient falls off steeply with the magnitude of the coefficient. Such a distribution of selection coefficients seems plausible, and is in any case similar to that which is claimed to be the case by the neutralist school.

The result obtained by Gilpin *et al.* applies to overdominant loci, treated deterministically. It may be worth pointing out that a U-shaped equilibrium distribution of allele frequencies will also be generated deterministically amongst loci at which the heterozygote is intermediate in fitness. Consider a locus with two alleles, *A1* and *A2*, whose frequencies are p and q respectively. If the selection coefficient and the degree of dominance are given, the rate of substitution will vary directly with the genic variance, pq. Since this is maximal at $p=q=\frac{1}{2}$, and is minimal when either allele is near fixation, substitution will proceed rapidly at intermediate allele frequencies, but very slowly when either allele is very common. If we imagine an ensemble of loci behaving in this way, it is easy to see that most loci will accumulate in the regions where the rate of movement of allele frequency is lowest, that is near $p=1$ and near $p=0$. In this way, the frequency distribution of allele frequencies at loci which are undergoing directional selection in an infinite population will be U-shaped at equilibrium.

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ONDRICEK *et al.* REPLY—Bell correctly notes a possibility we had not considered, namely that the commonly observed U-shaped allele frequency distribution is to be expected assuming that polymorphic loci are in the process of becoming fixed for a better allele; this is simply because the fastest rate for change of transient polymorphisms is at intermediate gene frequencies.

Two predictions follow from his model. The first is that the rate of evolution of a population is proportional to its level of heterozygosity. There are insufficient data to test this, but the common finding of high heterozygosities in physically stable environments (the deep sea[2,3] and the tropics[4,5]) is not auspicious. Nor is the occurrence of moderate levels of variation in a 'living fossil', *Limulus polyphemus*[6], consistent with such a theory.

Second, the transient polymorphism model would predict that frequencies in the middle range, say 0.7 to 0.3, should not be conserved; that is, if related species or isolated populations of the same species share polymorphisms with allele frequencies in this range, it would argue for balancing selection. Tenacity for intermediate allele frequencies seems to be rather common. For example, the *Lacerta* data[7] show that *L. melisellensis* retains two alleles at intermediate frequencies for phosphoglucomutase-2 on all islands larger than 0.05 km², and *L. sicula* shows the same pattern for esterase-4. Among four species of the *willistoni* group of *Drosophila*, the analogous pattern is observable for aldolase, xanthine dehydrogenase, and adenylate kinase-2 (ref. 4). The examples could be multiplied.

In summary, we think it likely that only a small fraction of polymorphic loci are transient, and in the process of becoming fixed for an overall 'better' allele.

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reviews

Mayr's evolutionary mission

A. J. Cain

Evolution and the Diversity of Life: Selected Essays. By Ernst Mayr. Pp. ix+721. (Belknap/Harvard University: Cambridge, Massachusetts, 1976.) £15.

THIS book is most likely to be reviewed by those for whom it is not intended (myself included). At first sight it is a strange collection of scientific papers (not essays like Julian Huxley's), whole or more usually excerpted, classed under the headings: evolution, speciation, history of biology, philosophy of biology, theory of systematics, the species, man (merely a blast—well aimed at the time—against the excessive taxonomic splitting of fossil hominids), biogeography and behaviour. Some, such as Mayr's paper on change of genetic environment and evolution (1954), propounding a theory of genetic revolution in peripheral populations, are major contributions to evolutionary theory at a professional level; others, now translated into English, are elementary expositions of neo-Darwinian theory for the benefit of continental audiences.

Useful, indeed sometimes excellent, studies of important but neglected or misunderstood biologists (Lamark, Louis Agassiz, Karl Jordan) are in the book, together with a ponderous (but correct) reply to Hennig's extraordinary ideas on evolutionary systematics, which could have been shortened, and tantalising snippets on topics as important as: accident or design (four pages); explanatory models in biology (five pages), and theory formation in developmental biology (six pages). The book is especially rich, as would be expected, in papers on speciation, the species and biogeography, although in the last only general discussions and conclusions are given, detailed documentation being left out. Throughout, papers have been adapted for this volume, but I am told that in every case where the author has changed his opinion this is duly noted between square brackets, so that the reader can see his intellectual progress. Mayr says himself in the general introduction that where he has included several essays on the same subject, this is done to "illuminate the maturation in my thinking". It would seem then that there is something in the book for everyone, although perhaps not much for some.

I think this is true. Mayr has a better acquaintance with European continental evolutionary biology than many westerners, and is probably the best judge of what is useful among his papers for the continental readers. Anyone in Britain or the United States might be inclined to dismiss his two papers for continental audiences as not worth reprinting, but the appearance of Grassé's book, *L'Evolution du Vivant,* (1973) makes one pause. If this is the best that the doyen of French zoology can do, then a vast amount of elementary instruction is needed on the continent before one can even start discussion. Although he has acquired some reasonably up-to-date references, Grassé is still well within the tradition set by Flourens's notorious attack on the *Origin of Species.* Some of the papers in Mayr's book are in journals not easy to come by, and more people than European continentals, in particular undergraduates and pre-doctoral students, will find the collection extremely useful. What is there in it for others?

Any evolutionist or historian of biology will be interested to see how Mayr's thought has changed since 1940, but he will have to work out the changes for himself, and must refer to other papers not included in it. I am very grateful to Ernest Mayr for outlining to me the principal changes (the notes that follow are, however, mine). In 1942, following the theory accepted then, he thought of polymorphism as neutral, but in 1945 (in a paper in *Lloydia* not, alas, included in the book) he had already come to see that selection must be acting. Formerly he regarded evolution as basically a change in gene frequencies but in the 1970s as a change in organisms, that in gene-frequencies being merely a consequence of selection on organisms. Formerly, he took the view that evolution would go fastest and speciation would be most frequent in big populations, but now he thinks that small peripheral populations, that have undergone a genetic revolution when they have been established, are the principal source of new evolutionary forms.

Coordinate with this is his strong emphasis on the genotype not as a 'bean-bag' of genes but heavily coadapted. In systematics, he points also to a sorting out of the ideas of the species as a taxon and as a category, previously confused in some of his earlier papers. In the highest levels of evolutionary theory, he is most impressed by the advent of population thinking, in which one is dealing with populations of interacting individuals, each of which is unique. It is clear from the book that he was profoundly impressed by a public encounter with Niels Bohr in which that great man seemed to misunderstand completely the greatest advance in biology. We have all met lesser men who enunciate such gems as "Molecular biology *is* biology, and all biology *is* molecular biology". Mayr has seen too much of the diversity of living things and the emergence of levels of organisation to be content with such a half-truth. He has always been well ahead in his appreciation of the complexities of natural selection, the enormous fluctuations in its rate, in short the biology of selection in the wild, although it took him some years (not documented here) to absorb the British work on the subject, and he apparently still puts faith in historic accident determining characters of great groups, and in the selective equivalence of isoalleles. This is not suprising as his own work has lain almost entirely in long-term evolution and the results rather than the action of selection.

This is a missionary book, against ignorance of the modern evolutionary synthesis, against the distortion of biology by molecular biologists, against the distortion of systematics by *a priori* considerations and against the neglect of major personalities in the development of evolutionism. Unfortunately, the style is not that of a missionary but a crusader, who believes that the only good heathen is a dead one. To be told that gradual evolution was accepted quite universally soon after 1859, that there is no such thing as mutation pressure (p 12) and yet that it has some direct results at the cellular level (p 111), is rather bludgeoning. Such remarks as "Thousands of experiments have proved that..." (no references), and "Discoveries in modern biology indicate that..." (no references) are more reminiscent of political propaganda. The missionary may have spoken more truly than he knew in saying (p 34) "Merely asserting [these claims] dogmatically will not convince those who, until now, have been disbelievers". It may even unsettle belief. What is the actual evidence not that genes interact but that genotypes are coadapted? □

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Plant transport processes

Transport in Plants. Vol. 3: Intercellular Interactions and Transport Processes. Edited by C. R. Stocking and U. Heber. Pp. xxii+517. (Springer: Berlin and New York, 1976.) DM145; $59.50.

THIS is the third volume covering transport process in plants in the new series of the *Encyclopaedia of Plant Physiology*. The first two volumes covered phloem transport and transport in cells, tissues and organs. This third multi-author volume is concerned with intracellular interactions and transport processes, a topic of considerable current interest which received little coverage in the first series published 1955–58. Indeed, as the editors point out, this is an area which is still poorly understood, particularly in relation to plants. This last point is illustrated by a glance at the references cited in many of the chapters, a high proportion being concerned with animal or microbiol systems. Thus, although this volume should become outdated fairly rapidly, it will serve as a useful summary of progress to date and as an indicator for future research.

The book is divided into four major sections concerned with membrane structure, intracellular interactions, transport in relation to energy conservation, and the theory of membrane transport. There is a little overlap with the second volume in the series in the first and last of these sections as regards membrane structure and the principles of irreversible thermodynamics. This is largely unavoidable and, in general, this volume complements the others very well.

The first section containing one chapter on plant membranes is disappointing. For example, the few pages on membrane isolation are really too superficial for an encyclopaedia; a number of the subsections are covered by reference to one or two reviews of work on animals. The treatment of plant plasma membranes could also be misleading. Lists of characteristics and markers are given, although these are based on only a handful of papers and are the subject of considerable current debate.

The second section of eight chapters covers intracellular interactions involving the nucleus, chloroplasts, the organelles concerned with photorespiration, mitochondria, vacuoles, and the endomembrane system. The chloroplast is particularly well reviewed in three chapters concerning re-cycling, export and storage in this organelle, metabolite carriers, and compartmentation and transport in C_4 photosynthesis.

It is clear that, although we know a great deal about the structure and separate biochemistry of various individual organelles, our knowledge of their transfer processes and interactions is still in its infancy. This is illustrated by the discussion of the importance of inorganic pyrophosphate in maintaining maximal photosynthetic rates in intact, isolated chloroplasts; it is thought to work by regulating the movement of orthophosphate into the chloroplast. Although this process probably is completely artificial, it indicates the role of the cytoplasm in regulating photosynthetic rates *in vivo*.

The importance of the treatment of chloroplasts after isolation, often given less attention than the isolation procedure itself, is stressed and deserves much wider consideration. The chapter on vacuoles further emphasises the difficulty of studying organelles in isolation from the cytoplasm, although the digestive lysosomal-like role of the vacuoles now appears to be well established.

The third section of three chapters is concerned with ion tranport and energy conservation and transfer in chloroplasts and mitochondria. Work on chloroplasts, in particular, provides strong support for the chemiosmotic interpretation in the great phosphorylation debate. The final section and chapter reviews the basic concepts used in the theoretical analysis of membrane transport processes.

The book is well illustrated and contains a thorough author and subject index. Although a number of the topics have been recently reviewed in other volumes, and in some cases by the same authors, they are here brought together in a single volume which, overall, lives up to the high standards set by the preceding numbers in this series.

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Point defects

Point Defects in Crystals. By R. K. Watts. Pp. vi+132. (Wiley-Interscience; New York and London, 1977.) $27.90; £16.50.

THIS monograph deals with the magnetic resonance and optical properties of point defects. The first six chapters are theoretical. They cover electronic states of defects with tightly bound electrons (rare earths, transition metals, the F centre), with loosely bound electrons (donors, acceptors, excitons), vibrational properties (localised modes, vibronic transitions, the Jahn-Teller effect), defect chemistry (equilibria, thermodynamic potentials), theory of experimental methods (the spin Hamiltonian, optical properties), and electrons in covalent and ionic crystals. But this is not a textbook; supplementation by more pedagogic texts will be necessary. And the level is too advanced and too specialised for undergraduates, though suitable for postgraduates.

The next four chapters are reviews of point defects in the group IV, III–V, II–VI and alkali halide crystals. Materials dealt with include Si, Ge, diamond, GaP and GaAs. The II–VIs and alkali halides are dealt with by considering the defects and their manifestation in the different compounds, rather than by taking each compound individually. The coverage is thorough, although the author does not claim that it is exhaustive. The latest references are 1974 to 1976, depending on the material.

Reviews are, however, sooner or later overtaken by events, and this has already happened in at least one material Dr Watts has covered—diamond. This illustrates a problem inherent in reviewing fields of research other than one's own. (From the references it seems that Dr Watts's subject is the II–VI compounds.) Although the picture he presents of defects in diamond is largely correct, it is incomplete. And the identification of one important defect, though generally accepted before 1972, is widely disbelieved now. A diamond specialist would be aware of all this. One wonders if any of the other chapters have the same deficiency.

Nonetheless, the book is a useful introduction to the spectroscopic properties of these materials, provided it is supplemented by attention to the current literature and to more specialised reviews.

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Volcanoes and eruptions

Volcanoes of the Earth. (Revised edition.) By Fred M. Bullard. Pp. 579. (University of Texas Press: Austin and London, 1976.) £20.

Volcanoes of the Earth is, as the dust jacket blurb states, a revised and enlarged edition of Bullard's *Volcanoes: In History, In Theory, In Eruption*, first published in 1962. It is a handsome volume, very satisfying to leaf through and to have on one's shelves. In contrast to the numerous cheaply produced textbooks that one encounters these days, this one gives the impression of being a *real* book, and one which the publishers seem to have spared no expense in producing. There are, of course, numerous text figures and photographs, most of them excellent, and even a section of colour photographs. Most striking, however, is the liberal use of white paper. Many diagrams have a whole page to themselves, and the text pages have six centimetre margins.

There are three main parts to the text. The first deals with the background to volcanology, the second covers the types of eruptions, and the third is rather a hotch-potch entitled "Theory, Cycles, Utilization and Environmental Effects of Volcanoes".

The core of the book is the section on types of volcanic eruptions, and it is here that its major strengths and weaknesses lie. Almost all of the useful parts of the book are concentrated in this section; detailed histories of individual volcanoes and eruptions. Bullard exhibits the evidence of much patient scholarship here. He has obviously tracked down and read a vast number of original references, including some very obscure ones. To take just one example, his account of the eruption of Mt Katmai in 1912 and the subsequent scientific investigations is unquestionably better than any other book in print. Many other authors have written about the same events, but they have based their accounts on secondary or even tertiary sources, thus blurring and muddying the facts. Having gone right back to the primary sources, Bullard's descriptions have a notable freshness and precision. Neither are they mere dry reviews. Bullard uses his narrative skill to present his material in a thoroughly readable, stimulating style.

Having said this, it is unfortunate that one has to look at the other, tarnished side of the coin. Bullard nowhere discusses adequately the criteria by which he recognises different types of eruption, and often seems to confuse a *type* of eruption with an individual volcano. Thus, chapter 9 on "The Strombolian Type of Volcanic Eruption" makes no mention of *any* volcano other than Stromboli. (About one-third of the chapter describes a visit made by Bullard and his wife to the island.) Similarly, chapter 10 on "The Hawaiian Type of Volcanic Eruption" deals exclusively with eruptions in the Hawaiian islands. In chapter 9, Bullard does at least begin with a few vague comments on the nature of Strombolian eruptions; in chapter 10, he prefers to discuss Captain Cook and the early history of the islands.

The book would have been strengthened by at least some discussion of modern work on classification of volcanic eruptions by granulometric analysis of pyroclastic material. This omission is characteristic of the text as a whole. The dust jacket proclaims that "Recent advances in heat flow studies and the concept of sea floor spreading and plate tectonics are incorporated to present a view of volcanology consistent with our present state of knowledge". This is emphatically not the case. In fact, the book has generally a somewhat dated atmosphere to it. Only a small proportion of the many works referenced were published in this decade. There are, indeed, two chapters on island arcs and plate tectonics, but they sit rather uncomfortably at the end of the book as rather unnecessary appendages, not well integrated with the rest of the text. They are competent enough reviews, but are unlikely to be of much value at present, when there are so many other good reviews around, and particularly because Bullard dampens any enthusiasm his readers may have for plate tectonics by implying quite clearly that he is not terribly impressed by it all.

In summary, *Volcanoes of the Earth* is an excellent source of facts on historical volcanic eruptions. As a review of modern volcanology it leaves much to be desired.

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Crystalline rocks

Evolution of the Crystalline Rocks. Edited by D. K. Bailey and R. Macdonald. Pp. ix+484. (Academic: London and New York, 1976.) £16; $35.

ALMOST half a century has elapsed since the appearance of Bowen's *Evolution of the Igneous Rocks* (1928). In that time major advances in both equipment and experimental techniques have led to a wealth of published experimental data, presenting the contributors with an unenviable task in writing a sequel to Bowen's work, within the confines of a single volume. Not surprisingly all aspects of the contribution of experimental petrology to the understanding of the evolution of crystalline rocks could not be covered, but the omissions in the igneous section, noticeably the basic and intermediate rocks, have led to a marked imbalance in the text.

The opening chapter on experimental methods offers a basic introduction for newcomers to experimental petrology. More detailed accounts of specific techniques can be found in the references cited. The choice of PT subdivisions in the metamorphic section has inevitably led to a certain amount of duplication. This has, however, resulted in some interesting insights into the differing criteria used by individual contributors in assessing the various conflicting sets of data (see Greenwood and Schreyer on the stability of Al_2SiO_5 polymorphs).

Newton and Fyfe give a comprehensive account of blueschist metamorphism (57 pages), but have rather scantily covered eclogites and granulites (10 pages). Greenwood offers one of the best sections, striking a very good balance between the major aspects of experimental petrology and its application to quantifying the intensive parameters in a range of metamorphic rocks. Schreyer has adopted a slightly different approach concentrating more on the phase chemistry aspects at high temperatures and low pressures.

The section on granitic rocks is mainly concerned with the crystallisation of haplo granitic melts. The reader's attention is drawn to the paucity of data for water undersaturated systems and the composition of the vapour phase in equilibrium with solidus reactions. In his concluding remarks, Luth has essentially critically reviewed his own contribution, by pointing out the omissions in dealing with the ultimate source of granitic melts and the volcanic rocks of granitic composition. Bailey presents a very concise review of the major aspects of alkaline rocks, delineating some of the problems in the genesis and subsequent crystallisation of the melts.

The imbalance in some of the contributions and the questionable policy of omitting certain major aspects of igneous experimental petrology will probably reduce the usefullness of this book as an undergraduate text.

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Atomic and molecular processes

Atomic Processes and Applications. (In Honour of David R. Bate's 60th Birthday.) Edited by P. G. Burke and B. L. Moiseiwitsch. Pp. x+533. (North-Holland: Amsterdam, New York and Oxford, 1976.) Dfl.165; $65.95.

THIS book forms a remarkable tribute to the scientific work in atomic and molecular physics over the past 40 years of David R. Bates' life. The scene is set in a perceptive and affectionate tribute to David Bates on his sixtieth birthday by Sir Harrie Massey, who begins by recalling his first meeting with Bates when, in 1934, he went to Queen's University, Belfast, as Lecturer in Mathematical Physics. Bates was one of the group of able young men from Ulster attracted by Massey to work in theoretical physics. The work based on their cooperation first at Belfast, then at University College, London, and later, also, in the school founded by David Bates when he returned as Professor to Queen's University in 1951, represents a large part of the progress made over this period in the study of atomic and molecular processes. It is indeed a striking example of the influence of the great leader and teacher in science that so many of the authors of articles in this book have been students at Queen's University. All of them have more or less close connections with either University College, London or Queen's. In this respect, it is not surprising that the book conveys an extraordinary sense of family.

Apart from the introductory tribute by Sir Harrie Massey, the thirteen articles which make up the book form a highly readable account of the present state of progress in the study of atomic processes. The first three articles deal with the Earth's atmosphere: M. Nicolet on Stratospheric Aeronomy, J. C. G. Walker on the Upper Atmosphere, and M. B. McElroy on Problems in Atmospheric Pollution. M. Nicolet considers the principal reactions first in an oxygen atmosphere, then in turn adding hydrogen, nitrogen and halogens. The theoretical problem of stratospheric ozone is resolved and the situation concerning minor constituents described. J. C. G. Walker provides an admirable summary of present knowledge of the chemistry of the E and F regions, stressing the importance in the understanding of the phenomena of dissociative recombination of molecular ions as the process for removing ions and electrons, as first suggested by Bates and Massey in 1947. M. B. McElroy, in an excellent account of man's impact on the global environment, pays tribute to the classic contribution of Bates and Witherspoon (1952).

The theme of molecules in interstellar space is taken up by A. Dalgarno, who deals with formation and destruction mechanisms for CH and CH^+. The problem posed for the abundance of CH^+ by the existence of molecular hydrogen in interstellar clouds, is considered. The process of di-electronic recombination of special interest in connection with the solar corona, is the subject of a theoretically interesting treatment by M. J. Seaton and P. J. Storey.

The remaining eight chapters of the book are devoted to an authoritative series of reviews of some of the main fields of study in atomic and molecular processes. Pioneering contributions were made in many of these by David Bates, and he has continued to contribute to them up to the present day. Photoionisation of atomic systems and electron scattering by atoms are the subject of masterly reviews by P. G. Burke and M. C. McDowell. B. L. Moiseiwitsch and A. L. Stewart contribute chapters, respectively, on negative ions and oscillator strengths. There is a thought-provoking chapter on computational methods by I. C. Percival.

The book concludes with three chapters on ionic recombination and atomic collision processes by M. R. Flannery, R. McCarroll, K. L. Bell and A. E. Kingston. Throughout this series of chapters, it is fascinating to follow the increased understanding which has come from the use of atomic models of increasing sophistication and from the growth of computational facilities. Another interesting aspect is the balance of success in appropriate régimes of quantal semi-classical and classical methods.

The editors are to be congratulated on the volume of work which they have brought together. The presentation is of uniform clarity and free, as far as I could detect, from all but the most trivial misprints. It must be a great joy to Professor Bates to have gathered together for him by this group of his students, colleagues and friends a description of a scientific edifice which he helped to found and did so much to build. Apart from this celebratory aspect the book is to be recommended as providing an admirable description of the state of the art in the study of atomic processes, which are of such increasing importance in a growing number of fields of interest in contemporary physics.

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Earth pictures

Mission to Earth: Landsat Views the World. By N. M. Short, P. D. Lowman, Jr, S. C. Freden and W. A. Finch, Jr. (NASA: Washington, DC, 1976.) $14.

LANDSAT, launched in 1972, was the first of a generation of unmanned satellites dedicated to repetitive surveillance of the continents and adjacent coastal waters to gather data for a variety of practical applications in Earth Resources disciplines, such as cartography, geological mapping, mineral resource evaluation, water inventory, coast and wetlands management, land-use assessment, and so on.

The above publication contains some 400 colour plates of outstanding Landsat pictures of areas all over the World, compiled and selected from more than 100,000 processed and reconstructed images, obtained during its first four years of operation. Each plate is accompanied by a carefully prepared narrative caption, summarising notable features. Taking into account that these photographs were available anyway from their Earth Resources program, it can only be warmly welcomed that NASA has made this selection of outstanding pictures available to the general public at the comparatively low price of $14. These photographs are likely to be of great interest not only to the various resource specialists amongst the appropriate Government authorities in the various countries concerned, but also to the serious teachers and students in geography, geology and allied disciplines, and to the general public with an interest in travel and geography.

Copies and slides of individual photographs are also obtainable, as well as free copies of an educational supplement, the *Educator's Guide,* which provides a general description of the Landsat program, the operation of the satellite and an explanation of remote sensing and imagery with suggested classroom uses of the material.

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Recent scientific and technical books

Chemistry

KONIG, E., and **KREMER**, S. Ligand Field Energy Diagrams. Pp.viii + 454. ISBN-0-306-30946-7. (New York and London: Plenum Press, 1977.) $59.40.

KRUUS, Peeter. Liquids and Solutions: Structure and Dynamics. Pp.x + 582. ISBN-0-8247-6427-7. (New York and Basel: Marcel Dekker, Inc., 1977.) SFr.145.

LADD, M. F. C., and **PLAMER**, R. A. Structure Determination by X-ray Crystallography. Pp.xvi + 393. ISBN-0-306-30844-4. (New York and London: Plenum Press, 1977.) $35.40.

LEE, Lieng-Huang (edited by). Characterization of Metal and Polymer Surfaces. Vol. 1: Metal Surfaces. Pp.xiv + 517. ISBN-0-12-442101-6. $26.50; £18.80. Vol. 2: Polymer Surfaces. Pp.xv + 465. ISBN-0-12-442102-4. $24; £17.05. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.)

LENK, R. Brownian Motion and Spin Relaxation. Pp. x + 250. ISBN-0-444-41592-0. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl.110; $44.90.

LEWIS, D. A., and **CHARNOCK**, P. (compiled by). Index of Reviews in Organic Chemistry: Second Cumulative Volume, 1976. Pp.308. ISBN-0-85186-539-9. (London The Chemical Society, 1977.) £6.50; $13.

LEVY, F. (edited by). Structural Chemistry of Layer-Type Phases. (Physics and Chemistry of Materials with Layered Structures.) Pp.viii + 392. ISBN-90-277-0714-6. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977.) Dfl.105; $39.50.

McAULIFFE, C. A. (edited by). The Chemistry of Mercury. (Aspects of Inorganic Chemistry.) Pp.vii + 288. ISBN-0-333-17863-7. (London and Basingstoke: The Macmillan Press, Ltd., 1977.) £25.

MARCHAND, Alan P., and **LEHR**, Roland, E. (edited by). Pericyclic Reactions, Vol. 1. (Organic Chemistry: a Series of Monographs, Vol. 35.) Pp.xi + 286. ISBN-0-12-470501-4. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) np.

MARCUS, Y., and **BEN-DOR**, L. (congress editors). Plenary lectures presented at the 25th International Congress of Pure and Applied Chemistry, Jerusalem, Israel, 6-11 July 1975. (International Union of Pure and Applied Chemistry, in conjunction with Israel Academy of Science and Humanities, National Council for Research and Development, and Israel Chemical Society.) Pp.73. ISBN-0-08-020952-1. (Oxford and New York: Pergamon Press, 1977.) £10.50.

MILLER, William H. (edited by). Dynamics of Molecular Collisions, Part B. (Modern Theoretical Chemistry, Vol. 2.) Pp.xv + 380. ISBN-0-306-33502-6. (New York and London: Plenum Press, 1976.) $39.50.

MILLICH, Frank, and **CARRAHER**, Jr, Charles E. (edited by). Interfacial Synthesis. Vol. 1: Fundamentals. Pp.xi + 298. ISBN-0-8247-6372-6. (New York and Basel: Marcel Dekker, Inc., 1977.) SFr.113.

NECKERS, Douglas C., and **DOYLE**, Michael P. PSI Study Guide for Organic Chemistry. Prepared by Erich C. Blossey. Pp.xxii + 246. ISBN-0-471-08345-3. (New York and London: John Wiley and Sons, Inc., 1977.) $6.85; £4.

NECKERS, Douglas C., and **DOYLE**, Michael P. Organic Chemistry. Pp.xxxvi + 1147. ISBN-0-471-63091-8. (New York and London: John Wiley and Sons, 1977.) £16.50; $27.90.

NEWTON, G. W. A. (senior reporter). Radiochemistry, Vol. 3. (A Review of the Literature Published during 1974 and 1975. A Specialist Periodical Report.) Pp.viii + 141. ISBN-0-85186-274-8. (London: The Chemical Society, 1976. Obtainable from The Chemical Society, Blackhorse Road, Letchworth, Herts; American Chemical Society, 1155 Sixteenth Street, N.W., Washington, DC.) £11.50; $23.

PARISH, R. V. The Metallic Elements. Pp.xii + 254. ISBN-0-582-44278-8. (London and New York: Longman, 1977.) £7.95.

PARKER, W. (senior reporter). Alicyclic Chemistry, Vol. 5. (A Review of the Literature Published during 1975. A Specialist Periodical Report.) Pp. ix + 439. ISBN-0-85186-612-3. (London: The Chemical Society, 1977. Obtainable from The Chemical Society, Blackhorse Road, Letchworth, Herts.; American Chemical Society, 1155 Sixteenth Street, N.W., Washington, DC.) £28; $56.

PATAI, Saul (edited by). The Chemistry of Double-Bonded Functional Groups. Part 1: Pp. xiv + 1-651. ISBN-0-471-99464-2. £29; $52.50. Part 2: Pp.xv + 653-1343. ISBN-0-471-99465-0. £29.50; $55. (The Chemistry of Functional Groups, Supplement A.) (:London and New York: John Wiley and Sons, 1977.) Supplement A, Parts 1 and 2 £58.50; $107.50.

PORTER, Roger S., and **JOHNSON**, Julian, F. (edited by). Analytical Calorimetry, Vol. 4. Pp.ix + 251. ISBN-0-306-35244-2. (New York and London: Plenum Press, 1977.) $33.

PULLMAN, Bernard, and **GOLDBLUM**, Natan (edited by). Metal-Ligand Interactions in Organic Chemistry and Biochemistry, Part 1. (Proceedings of the Ninth Jerusalem Symposium on Quantum Chemistry and Biochemistry held in Jerusalem, March 29th-April 2nd, 1976.) (The Jerusalem Symposia on Quantum Chemistry and Biochemistry, Vol. 9.) Pp.x + 396. ISBN-90-277-0751-0. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977.) Dfl.100; $39.50.

ROBIN, J. P. (colloquium editor). Plenary Lectures Presented at the XVIII Colloquium Spectroscopicum International, Grenoble, France, 15-19 September 1975.) (International Union of Pure and Applied Chemistry (Physical Chemistry Division), in conjunction with Groupement pour l'Avancement des Méthodes Spectroscopiques et Physico-Chimiques d'Analyse (GAMS). Pp.45-126. ISBN-0-08-021569-6. (Oxford and New York: Pergamon Press, 1976.) £4.45.

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obituary

W. Grey Walter

DR W. GREY WALTER, who had an international reputation for his work on brain function, died suddenly on 6 May 1977, aged 67.

William Grey Walter—Grey to his many friends—was born in Kansas City in 1910 of an American mother and an English father, Karl Walter, editor of the *Kansas City Star*. The family came to London in 1917 and Grey went to Westminster School and then to Cambridge where he obtained a first in natural sciences in 1931, and did postgraduate work on nerve physiology with Professor (now Sir Brian) Matthews.

In 1935 he joined Professor F. L. Golla at the Maudsley Hospital and started the work on brain function that was to occupy all of his working life. In 1936 almost by chance an opportunity arose to record the electrical activity of the brain (electroencephalogram, EEG) of a patient with a cerebral tumour, and the characteristic low frequency (1 Hz) activity was discovered. This work opened up a new era in the detection and localisation of brain lesions. using the EEG. During this same period Grey was recording the EEGs of many epileptic patients and showed that many of them were abnormal *between* fits.

In 1939 Golla moved to Bristol to open a research laboratory and clinic called the Burden Neurological Institute and took Grey Walter with him. Here Grey was able to expand his work and was responsible for many new developments and discoveries. He made the first instrument in Britain for electro-shock therapy (1939) and was co-worker in its early use; he opened up the first clinical EEG department in Britain (1940); he developed the first portable electroencephalograph and the first low frequency automatic wave analyser (1942); and he developed electrode probes for recording deep within the brain during neurosurgical operations (1941).

In 1943 he discovered the theta rhythm; and later used the stroboscope to activate brain abnormalities in epileptic patients, triggering the flash from the brain activity—a particularly effective form of bio-feedback. It was about this time that he investigated the relationship between the EEG and mental imagery; in 1947 he published a paper on the effects of sensory stimuli on the EEG. In the same year he was awarded the ScD from Cambridge University for his outstanding scientific work. Although a physiologist, his work was often closely related to clinical practice and in 1949 to mark the importance of this aspect of his work he was awarded an honorary MD and made honorary Professor of the University of Aix-Marseille.

At the turn of the decade Grey Walter was attracted to the new science of cybernetics which gave opportunity of rich cross-fertilisation of many disciplines. He developed several fascinating models of behaviour of which the mechanical tortoise *Machina speculatrix* was the most famous. In his book *The Living Brain* (1953) he drew together many aspects of brain science. Its value was not so much scientific (as most of the work had been published elsewhere) but as one of the first successful attempts to present such concepts in a readable form to the non-scientist.

From the beginning Grey Walter was fascinated by the concepts of Pavlovian conditioning and its application to learning processes. Many and varied were the experiments on this theme throughout the years using the EEG as an indicator of brain function in behaviour, but it was not until the technological development of averaging devices in the early '60s, enabling brain responses to sensory stimuli to be extracted easily from the background EEG, that the crucial discovery was made. This was the observation that changes of cortical potentials occur as a person having been prewarned by one stimulus makes a response to a second. This Grey Walter called the contingent negative variation (CNV) and published the work in *Nature* in 1964. Grey discovered the CNV not with the open mind said to be used by scientists but by a stubborn prejudice and persistent, perceptive searching for what "had to be there."

By a cruel and ironic stroke of misfortune, Grey Walter was involved in a road traffic accident in 1970 and suffered severe brain injury. He was unconscious for about three weeks but gradually made a remarkable recovery. He was appointed Emeritus Consultant when he retired from the Burden Neurological Institute in 1975. In the same year the EEG Society, of which he was a founder member, struck a special Grey Walter Medal and presented the first award to Grey Walter himself for his outstanding achievements. In 1974 he was awarded the Oliver-Sharpey prize of the Royal College of Physicians.

In these days of high technology it is difficult to realise the primitive nature of the scientific tools available in the 1930s. The electroencephalogram as recorded from scalp electrodes is about 50 microvolts peak to peak and has a frequency range from DC to about 100Hz. Even today much care is needed in order to record the EEG in a hospital environment where electrostatic and electromagnetic interference causes many problems. When Grey Walter started, the balanced (push-pull) amplifier had just been developed by Tonnies and the electronic components needed for the amplification of the low frequency brain activity were somewhat bulky when available. All the early equipment was made by Grey himself and had to be treated with loving care. Much of his early work was done using a smoked drum as recorder and many disagreeable hours must have been spent coating the paper; not until 1939 did he obtain a three channel inkwriter that gave long uninterrupted records. Much of this technological development occured during the war years when shortages of equipment were very acute.

The main significance of his early work was not technological, though this was impressive enough, but the introduction into clinical medicine in this country of a unique method of studying the *functioning* brain. Even with the introduction of the EMI scanner and computer techniques thirty years later the EEG remains the only non-invasive method of observing the brain in action on a second to second time scale. Perhaps not surprisingly, the application of these techniques to patients by a physiologist was not always welcomed by the medical profession and even today there are only one or two scientists without medical qualifications reading clinical EEGs.

But what of the man? Grey was generous and helpful to others, especially young scientists, but sometimes his behaviour seemed arrogant, especially with senior colleagues, in the way of many scientists when they know (or think) that they are right. The publication of the CNV was delayed for about a year while he made sure that it was not an artefact due to eye movements. Even after the publication, many groups throughout the world doubted whether it was a cortical event; Grey's attitude was firm, 'Let them spend time doubting, it will take them longer to catch us up.' He was unconcerned about money and in the 1950s was content with such a low salary that the income tax inspector did not believe his declared income. Yet over the years Grey obtained hundreds of thousands of pounds for the equipment and work at the Burden Institute. I think that he considered that to be able to work on brain research was a privilege that had to be paid for and I am sure that he would have continued working for nothing if the necessity had arisen. Work was a major passion in his life and he would spend many, many hours in the laboratory trying out new ideas often without any real lead to follow, persisting in the belief that discoveries are made in the laboratory and not at the desk or committee meetings. It is a pity that there are so few like him.

R. Cooper

Frank Green

Dr Frank Green, CBE, MD, who died on 30 April 1977 in his 77th year, had lived his professional life as a research administrator. From 1929 to 1955 he served the Medical Research Council rising through the various grades from Medical Officer to Principal and at the end of his period was Publications Editor. Their special reports were perhaps appropriately named "Green" books.

He worked under a succession of Secretaries from Sir Walter Fletcher and Sir Edward Mellanby to Sir Harold Himsworth. In the pre-war period of his MRC service the annual budget, allocated directly by the Privy Council, rose from approximately £150,000 to nearly £200,000. The organisation remained small, intimate and friendly and its relationships with a small band of research workers were personal, smooth running and inexpensive. Frank got to know them all and his wife Joyce acted as a charming hostess to many of us.

The MRC then occupied a pleasant old Georgian house at 38 Old Queen Street and the Council acted with an ease of judgement and the lightest of control. Research was still cheap, equipment often home made at laboratory benches, and grantees were trusted to spend their modest (often three-figure) allocations to the advantage of their experiments, which were, in those days, usually pursued with their own hands, with very limited technical help. When the War ended in 1945 the Treasury began to intervene in the allocations which in 1946 reached half a million pounds, and were subsequently multiplied to two million by 1955. The MRC was now growing into a larger and less informal body, personal contacts became less easy to maintain, and the channels of communication became more complex.

Frank then side-stepped into the growing Wellcome Trust thus coming back into a much smaller organisation, in which he was in continuous daily contact with the chairman, Sir Henry Dale, the doyen of medical science of our era. The Wellcome funds at that time were limited to a few hundred thousand pounds and decisions of great responsibility fell largely to Dale's admirable scientific judgement combined with Green's personal knowledge of research applicants.

The allocations in those days were mainly to provide for costly equipment such as electron microscopes and many new laboratories of which the Medical Schools were in dire need. Indeed with out help from the Wellcome Trust medical research in our Universities in the immediate post-war years would have been severely hampered. Frank as the Executive Secretary was thus a trusted and key figure approached by everybody in need of supplements. Extrapolating backwards through his experience of the MRC he had indeed been a key figure in facilitating almost the whole development of British medical research from 1930 into the present era. His experience as Editor in the MRC gave him a capacity to work hard on drafts and produce his reports in impeccable English.

He was frequently invalided, often crippled by bronchitis and asthma. He worked to the limit of his capacity until recurrent illnesses each winter made it difficult for him to continue with his growing responsibilities while other physical disabilities also made him less mobile. When he relinquished his advisory services to the Wellcome Trust he gave part time services to the Science Committee of the growing British Heart Foundation. Once again his knowledge of personalities and his experience in judging applications helped to create a system in which the expertise of the various committees was always supplemented by consultation with appropriate experts in collateral fields. He thus epitomised in himself the ethics of research development and support based on the highest judgement of achievement and an intimate knowledge not only of the applicants and their sponsors but also of suitable referees.

He endured a long physical illness with patience supported and nursed by his loyal and devoted wife who always welcomed visits from their old friends.

Sir John McMichael

M. F. Lucas Keene

Professor Mary Frances Lucas Keene, doyenne of British anatomists, was born 15 August 1885 and died 9 May 1977. Her professional life was dedicated to the Royal Free Hospital School of Medicine (formerly London School of Medicine for Women), to the interests of the medical profession and the enhancement of the status of women therein. A brilliant undergraduate in the School she attracted encouragement from Stanley Boyd and F. G. Parsons and after adopting a career in anatomy was later influenced by F. Wood Jones. She graduated MB, BS in 1911 and joined the School's anatomy department to serve it with distinction as Lecturer in Embryology and Senior Demonstrator of Anatomy (1914), Lecturer and Head of Department (1919) and University Professor (1924-51). On retirement she was elected Emeritus Professor in the University of London.

Her principal researches were neurological and produced meticulous studies of the posterior commissure, the subcommissural region, muscle spindles and intramuscular sensory organs: her pioneer investigations of the process of myelination earned the award of the DSc of London University.

Lucas Keene was exceptionally endowed with powers of organisation and judicial assessment and was wholeheartedly committed to duty. These qualities gained early recognition and were inevitably empanelled in the general service of School and University. Without remission of departmental commitment she accordingly assumed and discharged with notable success an onerous burden of scholastic and administrative business which involved, during the Second World War, added responsibility for the School's migrant preclinical departments and the reconstruction of its bomb-devastated premises.

Her integrity and profound common sense fortified by her personal dignity and charm, redounded to her School's prestige and engendered wide extramural appreciation of her judgement and counsel. Her achievements and services were fittingly recognised by her election to the presidency of the School of Medicine (1956-74), of the Anatomical Society (1949-51) and of the Medical Women's Federation (1946-49), to vice-presidency of the Medical Protection Society (1929-77), to the livery of the Worshipful Society of Apothecaries (1952) and to the Fellowship of the Royal College of Surgeons of England (1956).

A. J. E. Cave

nature

4 August 1977

Test ban: new hopes, new hurdles

DISCUSSIONS on a nuclear test ban covering explosions in all environments and of all yields have dragged on for very nearly twenty years, so the optimistic noises now being made following the Geneva talks between the Soviet Union, the United States and United Kingdom should not be misinterpreted as more than a ray of hope. Even so, prospects look better now than at any time since 1963 for this major arms control measure to be brought to a conclusion—if the thorny issue of peaceful nuclear explosions can be settled.

It was in 1958 that the first international scientific discussions were held on monitoring a test ban treaty, and these, together with a growing public concern about radioactivity, led to a 1963 treaty, widely but not universally signed and ratified, which outlawed test explosions in all environments except underground. At the time it seemed that it was only modest disagreement between the United States and Soviet Union concerning on-site inspections and unmanned monitoring stations which stood between the negotiators and a truly comprehensive ban. In hindsight, however, it looks most unlikely that the United States would have been prepared to go the whole way in 1963, as ratification might have been impossible in the face of a very strong nuclear lobby.

For the first few years after 1963 a comprehensive treaty seemed out of the question politically and militarily, although seismic monitoring methods were steadily improving. Then, when seismology seemed to be incapable of offering any spectacular further developments, worries started to be made known about the possibility of evasion (by firing a shot shortly after an earthquake, for instance). And the growing vigour of the Soviet programme of peaceful nuclear explosions caused some to wonder whether military tests might be made under the cover of civil engineering. The Indian explosion in 1974 helped convert many more to this point of view.

Against this background the United States and Soviet Union made a rather odd move by agreeing on a bilateral treaty restricting tests to be below a yield of 150 kilotons. This agreement was widely regarded as a poor substitute for a comprehensive treaty (its ratification hearings are still being held in Washington) and as a means of holding up progress on a comprehensive treaty for many years. But this was before President Carter.

It is clear that Carter attaches considerable importance to progress on a test ban. The team that the United States is fielding at Geneva is remarkably strong and contains several of those who were involved in the 1963 negotiattions and who have showed continued interest in fostering arms control. For the moment, at least, the Arms Control and Disarmament Agency is in the driver's seat.

The meetings so far, in embassies, have been outside the normal Geneva disarmament forum of the United Nations Conference of the Committee on Disarmament (CCD), but if last week's optimism prevails, at some stage the matter will presumably be passed to the CCD where a more multinational analysis can be made of the technical and political issues. There are, however, still stumbling blocks. The first is that the US Congress would have to ratify the treaty, and although the nuclear weapons lobby in Washington is not what it used to be, the Energy Research and Development Administration is not likely to give away one of its birthrights without much agonising.

Second, it is by no means yet clear that the peaceful-nuclear-explosions issue can be resolved. The United States has all but abandoned its Plowshare programme and is likely to ask the Soviet Union to do the same to eliminate suspicions of misuse. The Soviet Union may find this unacceptable—a large portion of its Geneva delegation consisted of peaceful-explosions experts, which shows the importance it now attaches to these operations.

If the Soviet Union really wants to go ahead with its programme then the United States should not be allowed to get away with an excuse for stepping back from a treaty. Maybe the United Kingdom delegation should be hard at work trying to get an international agency to provide the necessary explosive. This idea has been talked about in recent years at the International Atomic Energy Agency but as yet no-one has asked them to look at the Soviet problem, which is undoubtedly a very complicated one. This is a job the United Kingdom should take upon itself. ☐

What kind of food policy?

Colin Blythe and **Howard Rush** examine whether nutritional goals can be a basis for agricultural policy

PARTICIPANTS at a number of recent conferences in Britain have tried to clarify the links between nutritional requirements, food production and supplies. Titles of meetings reflect the interest: 'People and Food Tomorrow', 'Towards a Healthier Britain' and, most recently, 'Nutritional Aspects of Food and Agricultural Policy in the United Kingdom'. All have raised a number of scientific, ethical and practical problems, and it has become apparent that until these are resolved little progress towards one coherent policy, integrating nutritional considerations, food supplies and agricultural production, seems possible. It is even argued that British policy makers remain unconvinced of the need for such a policy. What are the main arguments?

The possibility of malnutrition in some low-income groups has occasioned some concern. Clinical evidence of deficiencies is not common, however, except in certain well-defined groups, and where deficiency malnutrition exists, it is seldom due to simple poverty. Low levels of vitamin D and associated rickets occur in parts of the UK Asian community. Osteomalacia and vitamin C deficiency (not necessarily together) are found in a proportion of the elderly. Iron deficiency anaemia is said to affect about 10% of women in the UK, and folate deficiency (showing as megaloblastic anaemia of pregnancy) has occurred recently in about 2.5% of women. Deficiencies of other micronutrients may occur but the number of affected individuals is small.

In contrast, reports and papers dealing with the 'diseases of affluence' are growing more numerous; since 1970 nearly twenty reports have come from national bodies on the subject of coronary heart disease alone. As well as cardio- and cerebro-vascular disease, the list includes diverticulitis, appendicitis, constipation, certain forms of colonic cancer and, with its numerous sequelae (backache, arthritis of knees and hips, gallstones, renal failure and fallen arches), obesity.

The consensus of opinion in these reports is that some aspects of Western diet are partly responsible for the present levels of these diseases. Most conclude that wholesale changes in diet are necessary before the major causes of illness and premature death are brought under control. The US Senate Select Committee on Nutrition and Human Needs, under Senator McGovern, has recently promulgated a set of Dietary Goals: these include a reduction of total fat intake (within which there should be a decrease in the proportion of saturated fats and an increase in the proportion of polyunsaturated fats), a reduction in dietary cholesterol, sugar and salt, and an increase in consumption of complex carbohydrates. Sweden has had a Diet and Exercise Programme, incorporating similar goals, since 1972. Norway recently announced its intention to use pricing policy as well as education in an attempt to influence eating habits.

Evidence contested

Unfortunately, the factual evidence underlying goals of this type is contested. Disagreements over both causation and remedial measures are one reason. They have meant that the connection between diet and disease is not yet strong enough in the minds of UK policy makers for them to accord nutrition a priority in economic decisions. But having no policy amounts, in reality, to a policy of letting things drift, something which is clearly unsatisfactory. And the World Health Organisation said in 1969 that the incidence of certain diseases (coronary heart disease, for example) had reached almost "epidemic proportions".

The issues of causation may not have been resolved by clinical procedures, but then many of the tests needed to establish a causal relationship can only be performed on live human beings, and large numbers of them at that. Absolute proof may be years in coming. In the absence of absolute proof, this demands the exercise of scientific judgment based upon the best information avilable. Whether to wait for final proof (one way or the other), or whether to advise action now, is something which presents nutritionists with difficulties of judgment and also of conscience.

Camera Press

In Britain, the existing government role concerning diet is primarily one of monitoring, testing and education. In addition, a web of legislation governing food standards, additives, labelling and advertising has evolved largely on an *ad hoc* basis. Policy questions, such as whether Britain should or could feed itself, usually turn upon issues of security of supplies and the balance of payments. Seldom, if ever, are variations in food supplies or self-sufficiency levels related to their possible effects on the nutritional status of the population. Nor would it seem that nutritional considerations have prompted recent government interventions in food prices. Mollifying consumer unrest, assisting the economically disadvantaged and dealing with farm surpluses have been of greater concern.

The lack of nutritional reasoning behind adjustments to food supplies and prices does not, however, mean that such moves lack nutritional impact. EEC policy, for instance, is to reduce the price of certain items currently in over-supply, like butter, in order to stimulate demand, increase consumption and clear the surplus. Beyond boosting consumption artificially, this produces a secondary effect: the tendency to maintain isocaloric balance means that, as consumption of some foods rises, consumption of other items falls. In other words, by manipulating food prices for basically agricultural purposes, governments can apparently exert an effect on eating patterns and, unwittingly, on nutritional status. As recent EEC policy has sought to encourage consumption of some items which epidemiological evidence has linked with disease, the anxiety of many nutritionists to see nutritional criteria built into food policy becomes understandable.

Basis for policy?

But if the factual evidence underlying nutritional goals is controversial, so is the advisability of using them as the basis of policy. Government intervention in dietary matters is a hotly contested issue. Among nutritionists a sizeable lobby believes that the scale of diet-related disease is such as to justify a far more radical approach to public health. This group would like to see

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pricing and educational measures employed as part of a coherent policy of trying to prevent ill health, rather than attempting to cure the results of unwise living. A shift to preventive medicine is economically attractive. Although few figures are available for Britain, recent estimates put the total costs to the US economy of heart disease and other diet-related illness at between $30–35 billion annually. Costs to the UK economy must also be considerable. It may be that prices of alcohol, tobacco and some food items should more accurately reflect the community health costs incurred by their overconsumption.

A national programme of preventive medicine would almost certainly involve individuals assuming greater responsibility for their own health and basing their actions on adequate information. Attempts to educate the public on dietary matters, however, have a poor record of success. If changes are to occur in consumption levels public education may need to be reinforced with food price adjustments. Such procedures would have to be thought out with great care. Some evidence exists for suggesting that subsidies do not always benefit target groups as intended. So if taxes are also to be used to persuade consumers to alter their habits, steps will have to be taken to ensure the adequacy of diets among low-income groups.

Opponents of blanket government measures believe that improved screening and diagnosis are all that is required. After diagnosis, those at risk can take remedial action and those unaffected can escape being troubled or inconvenienced by wholesale measures. This view contends that, although nutritional needs can be assessed case-by-case, prescribing average requirements for entire populations on present knowledge is premature, and might even endanger some people's health. In discouraging overconsumption, it is asked, how is discrimination avoided against those who are not overconsuming, or those who, if they are at risk at all, may be at risk from under- rather than over-nutrition? And even if causation could be proved and remedial measures agreed, what of the freedom of individual choice? Is it for governments to coerce people into taking measures for their own good?

In the end, other factors may dictate the best course of action—the state of the economy, for example, or the adequacy of diagnostic facilities and personnel. Identifying every individual at risk of heart disease or stroke would involve a major programme of screening, with those placed in the 'at risk' category being examined at, say, yearly intervals, the rest perhaps every four or five years. Such a programme would add greatly to the costs of a Health Service already widely regarded as economically burdensome.

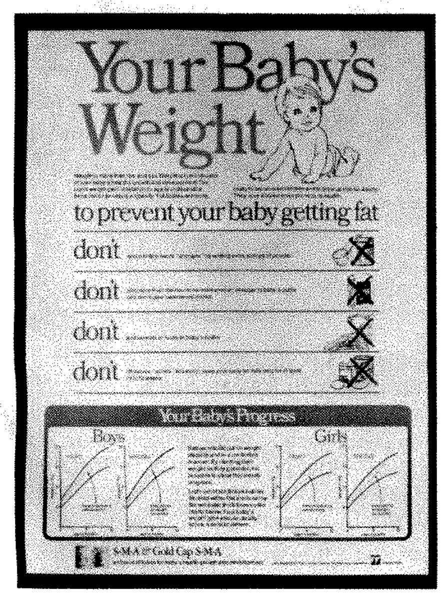
Disagreements

This discussion of means obscures the fact that no policy maker can be expected to act until a basis for action has been agreed. Unfortunately, there are strong disagreements over appropriate remedial measures. One example of conflicting advice to the government lies in recent evidence to the UK House of Commons Expenditure Committee considering preventive medicine. In this, the British Dietetic Association suggested that "a moderate reversal to diets in which bread and potato consumption are *increased* would be an advantage"; the British Medical Association felt that "the right diet for optimal weight was relatively high in fat, high in protein and *low* in carbohydrates". Such advice is just as confusing to individuals who wish to follow sensible eating regimes.

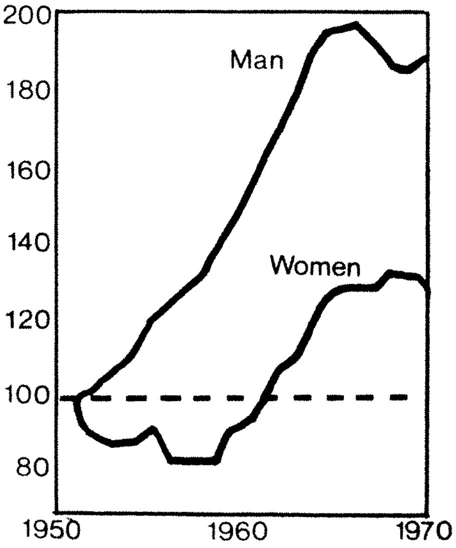
The quality and relevance as public guidelines of some other information is also open to doubt. Tools long accepted for evaluating nutritional status, such as the Recommended Daily Intake (RDI) tables, have begun to be questioned. Should the RDIs relate to a level of intake designed to secure optimum health (a concept in itself difficult to define), or to a level necessary to avoid the onset of illness? If the latter then, given that nutrient requirements vary not only for age and sex but also for bodyweight and metabolic type, illness for whom? The question of how much information now available can usefully be applied in constructing diets is also relevant. For example, is the RDI of 30 mg vitamin C per day for an adult to be secured by eating one tomato, or two? If minimum nutrient requirements cannot be assessed except on a case-by-case basis, if the notion of an 'optimal' intake is unscientific, and if the RDI tables offer no guidance on the designing of meals, what useful purpose is served by such information?

All these general problems have meant that governments have received little in the way of useful advice regarding the effect of agricultural policy on nutrition, although the fact that agricultural policy can influence nutrition is not seriously disputed. Clearly, when the major problems within nutrition are sorted out, it will be time for some cross-fertilisation to take place between disciplines. At a conference organised by the Nutrition Society in Reading recently, one example did emerge of the type of thinking that may be required to bridge the agriculture/nutrition gap.

In it, Professor W. Holmes briefly suggested some changes and problems facing UK agriculture if food goals outlined by the US Senate and others are to be realised. For example, an increase in the contribution of bread grains, vegetables and fruit to the diet, and a reduction in the intake of animal products (especially animal fats), might require a corresponding increase in the arable area and a reduction in the use of grain for animal feed. Though noting that such a change was technically feasible, he pointed out the considerable effects on the cost and profitability of farming and on the ancillary industries. A detailed study of the complex components within the farming community and of the incentives and advice necessary to achieve improvements was necessary, before any attempt to implement a food policy was likely to be successful. □



Where nutrition begins:
Blurb for mothers and others



Graph showing percentage increase in death rates due to coronary heart disease of men and women in England and Wales in 35–44 age group

Research on research

The UK Social Research Council (SSRC) has approved a grant application from the Science Policy Research Unit (SPRU) of the University of Sussex to assess the criteria used by the Science Research Council (SRC) for capital projects in basic science. Chris Sherwell reports.

IT WAS perhaps inevitable. Certainly an investigation of the funding of big science in Britain had everything going for it. Hard economic times produce whipping boys, and in Britain's research community it is big science. Likewise growing disciplines generate new fields to conquer, and for the social sciences in Britain science policy is indisputably one of them. Last week's news that the SSRC is funding research on the SRC's funding of research should therefore have been no surprise; in fact the fall-out may be more than inconsequential.

No one is denying that the SSRC has the right and duty to sponsor policy research. And no one doubts the SRC is a good candidate for consideration in the field of science. It takes a major (54%) but now declining share of the £216-million science budget doled out to the research councils. Similarly, with high energy physics, astronomy, space and radio science—the traditional areas involving large capital-spending projects—consuming some 60% of that share, big science is a natural object of attention. Indeed, in annual spending terms it is comparable to research and development on the fast breeder reactor or, in its day, on Concorde.

But there are some raised eyebrows, both inside and outside the SRC. Scientists who privately frown on some of the projects sponsored by the SSRC are never too difficult to find, of course; and the SRC cannot comment officially on projects sponsored by other research councils. But some reactions last week were sceptical. The director of one big science laboratory said rather obviously that he would have preferred to see the money going to his own work. Others, however, doubt the motives behind the proposed research. There were also questions about whether the project could even be done.

The proposal, a year in gestation, is contained in a document running to 18 pages with 5 appendices. It was submitted by Dr Roy Turner and Mr Keith Pavitt of SPRU and Dr Norman Dombey, reader in theoretical physics at Sussex, to the SSRC's political science committee. The SSRC approved the grant application in principle almost three weeks ago. The amount involved is £26,345 over two years, and the team is now looking urgently for a scientist and a social scientist so that the work can begin in October.

Their case for the study is simple. There is not as much work in Britain on the problems of science policy as there ought to be compared with North America. The SRC disposes of a large sum of money on basic research, and now faces hard choices distributing resources between big science and the rest of science. Yet, says the team, it is not known whether there is any technological benefit from, say, a high energy physics programme which could not have been achieved more cheaply by direct industrial research; nor are there guidelines on what proportions of funds should be allocated to fields in which there will be no foreseeable technological benefit.

The team says it has four aims. The first is to assess the research of six SRC centres in terms of certain criteria. The six centres are in high energy physics (CERN, Rutherford and Daresbury) and in astronomy (the Isaac Newton telescope, the Nuffield radio astronomy laboratories and the Mullard radio astronomy observatory). The criteria are 'intrinsic' (by which is meant the merit of the science and the researchers, and the benefits to other fields of science); 'educational' (the benefit to those who teach and those who work in other scientific fields); and 'technological' (the long term and short term application of the results and methods of research).

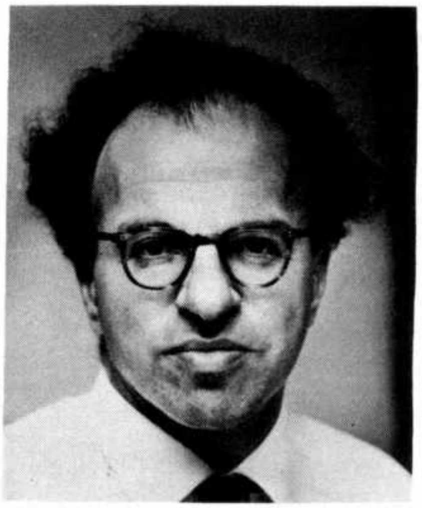
The team expects to spend two-thirds of its time on this aspect alone. The rest will be spent comparing the actual performance of the centres with their original justification, applying what is learned to proposals to set up new centres (the team has in mind the high powered laser facility and, though it is not in the SRC's remit, JET, the European fusion project), and finally considering British and international practice in funding big science.

In its proposal the team explains how it plans to proceed, and it is here that the difficulties may emerge. They will assess the research by speaking to scientists in the field, circulating questionnaires and examining published papers. The original justification for the centres will be

Bondi goes to Energy

AS WIDELY expected, Sir Hermann Bondi is to replace Dr Walter Marshall as Chief Scientist in the UK Department of Energy. For the past six years Sir Hermann has been Chief Scientific Adviser at the Ministry of Defence. He goes into the Department of Energy with no strongly expressed views on the record concerning choice of reactor, the balance between nuclear, hydrocarbon and renewable energy sources, or the wisdom of a fast-breeder programme; he freely admits that he has a lot to learn.

Sir Hermann looks back on his time at the Ministry of Defence with satisfaction; in part his role has been as a person to whom anyone could turn for disinterested and friendly advice and criticism. But he has always preached mobility—sometimes to a rather hostile audience within the civil service—and he clearly practises what he preaches. In his own words, the defence post is the "fourth good job I have thrown up". The others were a lectureship and fellowship at Cambridge, a professorship at Kings



College, London, and the directorship of the European Space Research Organisation. He admits there is a touch of masochism in this, but he felt he was getting too comfortable at Defence and was no longer an outsider. It will be interesting to see whether the Ministry of Defence has the courage to appoint another outsider.

learned, the team says, by interviewing those involved in the decisions (like Sir Alec Merrison and Sir Denys Wilkinson in the case of Rutherford and Daresbury and Sir Martin Ryle in the case of Mullard), from published sources (in the case of CERN) and if possible through access to original documents.

But the team is likely to face a critical problem over access to SRC documents. When Mike Gibbons and John Hartland did their study of CERN for the old Council of Scientific Policy (CSP)—a study the team mentions—the SRC gave special dispensation because the study was confidential to the Council; when Margaret Gowing sought SRC documents for her study of CERN on behalf of CERN, the SRC was prepared to allow access but the Department of Education and Science refused under the thirty-year rule. On that history, access is likely to be denied to the SPRU researchers.

The team does not see possible denial of access as a special difficulty. But Gibbons thinks otherwise. Not only did it take him and Hartland two years to do their study, which was of one centre, not six plus two new ones; they also found the original documents essential in providing what he calls a "well constructed net", the holes of which could be plugged with the help of interviews. Only with the documents could they appreciate just how much memories failed with time and how beneficial hindsight could be.

Whether or not the research yields controversial findings, the fact that it is going ahead at all has certainly provoked interest. The SSRC has some £25,000 at its disposal for science policy studies; this used to be in the hands of the CSP but was transferred to the SSRC when the Advisory Board for the Research Councils (ABRC) began. This award to the SPRU team means that other applications are denied. That is nothing new, except that, in going to one large project, it is ironically favouring 'big science policy' proposals at the expense of others whose merit may be as clear. More interestingly, and particularly as the research is about the SRC, the SRC is certain to have seen a copy of the proposals at an early stage and to have passed a verdict on it.

Two factors indicate why the SRC might be unhappy about it. One is the precedent of the Gowing case: lack of access is bound to reduce the value of the research. The other is its natural misgivings about the quality and partiality of science policy research, using anecdotal evidence or not. Indeed, Norman Dombey's own article on CERN, in *The Times Higher Education Supplement* earlier this year, is unlikely to have endeared this project to the SRC. And Keith Pavitt's views on the use to which Britain's scientists and engineers are put (typically, into aircraft, nuclear reactors and weapons instead of motor cars and machinery) won't have helped.

The topicality of the subject may have assisted the SSRC in reaching a decision. But one suggestion being put about last week, which could not be confirmed, was that the SSRC's political science committee regards its support for the SPRU proposal as a test case in its efforts to establish itself as the body sponsoring research that monitors decisions in government. The project, it was suggested, is a useful addition to the pressure for a lifting of the thirty-year rule.

As far as Dr Saul Rose, the committee's chairman, is concerned, there are bound to be differing views of its support for the project, as there are for much of the work it sponsors. As for projects that could "come up against a stone wall" because of lack of access, it was always hoped that something could be worked out; in any case projects were monitored, and could be called off or perhaps reshaped if they were unlikely to achieve the intended aims.

In the end, though, the project will be judged by its results. No one wanted to anticipate those last week, or to predict their likely impact. But one former high energy physicist privately expressed his reservations. The big science community in Britain, he said, was now highly sensitive to criticism after the financial battering of recent years. Though prepared to admit that the findings might be interesting, he couldn't see what useful purpose the project would ultimately serve. □

Reactor choice: last lap looms

The final and most important phase of Britain's tortuous debate over which type of thermal nuclear reactor should follow the existing Magnox generation is just beginning. Last week the Department of Energy (DEN) published extracts from the assessment made over the past nine months by the National Nuclear Corporation (NNC) of the three thermal reactor systems under scrutiny—the Pressurised Water Reactor (PWR), the Advanced Gas-Cooled Reactor (AGR) and the Steam Generating Heavy Water Reactor (SGHWR).

At the same time the Health and Safety Executive published an account from its Nuclear Installations Inspectorate (NII), responsible for nuclear safety, of its study of the generic safety issues of PWRs. And the DEN also made available a summary of the discussions on nuclear energy policy which emerged at the closed weekend seminar it held in May.

The nuclear industry, the customers, the politicians and others are expected to digest the contents of the massive NNC and NII volumes during a quiet August; discussions in September may then allow the matter to be put before the Cabinet for collective decision sometime in October. Mr Anthony Wedgwood Benn, the Secretary of State for Energy, for one, is looking forward to an interesting debate.

The NNC report says a decision has to be taken now both about the reactor systems and about actual orders "if the nuclear power option is to be maintained in a meaningful way". It says categorically that "there is no case for the adoption of the SGHWR" because there could be no operational experience of commercial sizes for a decade, there is no chance of exports and it is the most costly of the three.

Over the PWR and AGR, the report offers three options: continue with AGR, do not adopt PWR; adopt PWR, finish the current AGR programme but don't add to it; and adopt PWR, continue with AGR. The NNC says it cannot advise the first course because it would be rejecting important export opportunities; to be grasped, moreover, these require a decision now. The second course, it says, would help exports and lead to "the cheapest available electric power for Britain", but construction of the first PWR could only start in 1980; for the course to be acceptable, ways must be found of "maintaining the confidence and enthusiasm" of those laid off by the lack of AGR work.

The third course offers the advantage of keeping open an option, but has the disadvantage of dividing available resources. "In practice", the report suggests, "it is not likely to be sensible to maintain an ordering programme for two systems beyond the early 1980s". The summary concludes that the early adoption of the PWR would have to be supported by a domestic ordering programme of more than one reactor, and that an AGR station would have to be ordered and built, if the purposes of the choice are to be achieved.

The choice of thermal reactor was also the subject of a session at the nuclear policy seminar. Although Sir Alan Cottrell, former government science adviser, disagreed, both Ned Franklin of the NNC and Walter Marshall, the DEN's former chief scientist, thought safety arguments were not a significant factor in the choice, a point borne out by the NII report, which concludes that "there is no fundamental reason for regarding safety as an obstacle" to choosing the PWR.

One interesting contribution to the seminar came from Sir Hermann Bondi, however, whose appointment to the DEN's as chief scientist was confirmed last week. It was very likely, he said, that the first major accident would be with a PWR because of their widespread adoption; there could be some advantage, he said, in being outside the main stream. As in the NNC report, the seminar summary also reveals clearly the importance attached to the matter of exports in reaching a decision.

Chris Sherwell

BRITAIN

● British industrial journalists were almost buried under an avalanche of figures last week when four nationalised industries published their annual reports. The British Gas Corporation, the Electricity Council, the Central Electricity Generating Board (CEGB) and the Post Office all reported healthy profits. The achievements of the 'energy' industries attracted much attention. In spite of increases in the price of coal and oil and the slow increase in electricity demand, the Electricity Council and the CEGB made profits of £207 million and £130 million respectively. British Gas recorded a £31.5-million profit.

Of major concern to all three is the so-called 'energy gap' expected in the 1990s when North Sea oil and gas become scarce. Each report gives it prominence, and of course it greatly affects both short and long term research programmes. Last year British Gas spent £18.2 million on its R&D programme, the CEGB £11 million and the Electricity Council £41.7 million.

For the CEGB and Electricity Council nuclear power holds the key. But decisions over development to the year 2000 have yet to be taken, so neither has planned a detailed programme. Both, however, are improving existing reactor designs. Research is going on into the possibility of extending the life of Magnox reactors, and the CEGB is pushing ahead for the early operation of advanced gas reactors (AGRs) after the Hinkley Point Station became fully operational earlier this year. Research on the steam generating Heavy Water Reactor (SGHWR) will not increase; but the CEGB has increased its involvement in uranium exploration, and has signed agreements for joint exploration with Conwest Exploration in Canada and Urania Exploration in the USA.

Both the Electricity Council and the CEGB have faced a decline in the growth in energy demand. In view of the uncertainty this brings, the CEGB is giving priority in its more immediate research programme to improving the efficiency and safety of present plant designs rather than developing new plant with an unpredictable future. Taking into account all power stations currently under construction, it foresees no need for further new plants until 1985.

Meanwhile there is little British Gas can do to make gas supplies last into the 1990s except look for new fields and improve efficiency. Last year, as part of its energy conservation programme, it awarded the first Gas Energy Management Award, set up to recognise better use of gas in industry. It is already supporting research to produce natural gas from coal and oil. Last year it successfully manufactured on a commercial scale a new catalyst—CRG-F—for producing substitute natural gas from oil. And at the end of March, a slagging gasifier development programme to make gas from coal, sponsored by an American consortium, came to a successful end.

This latter side of the British Gas research programme overlaps with research done by the National Coal Board (NCB). Sir Derek Ezra, NCB's Chairman, outlined the NCB's £30-million five-year programme for developing new coal technologies when opening an exhibition at the Coal Research Establishment last week. Among its priorities is the manufacture of hydrogen and substitute natural gas from coal.

Perhaps the most surprising figures were those for the Post Office. With a profit of £291.3 million, it had almost doubled the previous year's figure and had succeeded for the first time in ten years in operating its postal service profitably. Of the £51.5 million spent on R&D, the lion's share, £50 million, went on telecommunications. The biggest single project, 'System X', was supported to the tune of £12 million. This is the Post Office's comprehensive telecommunications system of the future, and if it is to be fully developed then the percentage of the Post Office's resources spent on R&D will have to increase.

●After seven weeks of intense examination, British Nuclear Fuels Ltd (BNFL) finished making its case to the Windscale public inquiry last week. Since 14 June fifteen witnesses for BNFL have answered questions on the company's application to extend its reprocessing facilities by building an oxide fuel reprocessing plant. The key issues have been safety, the possibility of plutonium diversion and nuclear proliferation, and BNFL has by and large appeared in a good light. Now it is the turn of the Cumbria County Council and government departments to take the stand.

Just as its case finished, BNFL published its latest annual report. Although it alludes little to the inquiry, the report does mention the effect of the uncertainty surrounding future reprocessing facilities on the renegotiation of some contracts.

In his statement Sir John Hill, the company's chairman, speaks of increasing political constraints on international nuclear trade and economic difficulties in the UK and other trading nations. Still, the figures show BNFL managed to increase its sales of nuclear fuel services to £113 million last year, making a trading profit of £13.5 million, about £1 million up on the previous year. Exports contributed £11.1 million of this, down over £1 million on 1975–76. But there is little worry that the company will not have the money to carry out its investment programme: last year it successfully negotiated a loan agreement for £100 million with a consortium of British and foreign banks.

Ways have to be found of storing fuel as long as possible, especially if it cannot be reprocessed. BNFL is working in close collaboration with other countries on a vitrifying process to solidify liquid fuel for storage in water-filled ponds. The design and construction of a pilot vitrification plant is already under way, but the decision to build a production-scale plant will not be taken before BNFL's work is compared with that overseas. By the end of March, the company had set aside £13 million to pay for the vitrification in the late 1980s of all waste produced since the company was established in 1971.

Another of BNFL's activities which must expand to meet demand is the centrifuge enrichment of uranium. The first centrifuge enrichment plant started operation at Capenhurst last year and has already attracted £1,000 million-worth of business. The company also gained export orders for the conversion of enriched uranium hexafluoride to uranium dioxide using its Integrated Dry Route process.

Judy Redfearn

JET

Delayed again

The Council of Foreign Ministers of the European Economic Community (EEC) met in Brussels last week. Down for decision was the disputed site of the Community's fusion project, the Joint European Torus (JET). Chris Sherwell reports

HEADS of government may make history, but in the EEC they don't appear to determine it. At their meeting in London at the end of June, they insisted that the Council of Foreign Ministers should decide the site for JET at its next meeting. In the event the Council failed to agree. And a research ministers meeting set for two days later was cancelled.

Although the UK Foreign Secretary, Dr David Owen, said last week that he was "very disappointed" by the "non-decision", British newspapers had carried well-briefed reports on the day the meeting started warning that no decision could be expected. This did not apparently contradict earlier reports of a majority in favour of Culham: the majority remained, but only belatedly was it realised that West Germany was sufficiently committed to Garching not to want the embarrassment of an immediate climb-down.

The majority itself was a change from the dark days in March when Culham, or rather Britain, fell from grace. Since then Britain has made some conciliatory gestures, notably in removing her reserve on the EEC's joint research programme, which have helped to restore the site to favour. Britain has also changed tack in her arguments, giving greater emphasis to her concern over the lack of any Community research project in the country (Germany has three) while at the same time acknowledging that Garching is scientifically as suitable a centre to take JET as Culham.

But equally the West Germans have clung tenaciously to Garching. In Britain this is seen in straight political terms: Garching is in what is called 'Strauss country', a reference to the powerful leader of the Bavarian Christian Socialist Union whose influence on potentially shifting political coalitions is so great. An alternative view, that the Germans entertain worries about a Community project going to Britain when the Labour Party is shaping up once more for internal combat over the EEC, is dismissed: it is said that the West Germans know from experience not to doubt the commitment to the EEC of Labour's leaders. Certainly if this was an important factor, it might have been expected to have caused problems across a far broader Community front than just JET.

Not that JET is unimportant. The foreign ministers have now removed the subject almost totally from the hands of the research ministers, whose meeting last week was arranged mainly to clear up loose ends once a decision was taken. But the foreign ministers still only considered the subject informally. Their new president, Henri Simonet, himself a former EEC Energy Commissioner and now Belgium's foreign minister, spoke individually to ministers, taking his own private poll. The subject was then discussed over lunch.

No vote was taken, but it did emerge that if JET was not to go to Culham, it might not go anywhere—a possibility reportedly canvassed by the French, whose own fusion research (notably on Torus II) has been held up by the indecision over JET. Equally, however, if a vote was taken, it would apparently have gone in favour of Culham. One report had it that four countries favoured Culham, two favoured Garching, two were prepared to go with the majority, and one did not pronounce. But that accounting is probably only a 'guesstimate', at best.

Most affected is the JET team itself, based at Culham. Indeed, the lack of a decision is all the more surprising for the fact that the head of the comparable fusion project at Princeton in the United States sent a telegram ahead of the meeting to Paul Rebut, head of the JET team, and Donato Palumbo, director of the EEC fusion programme. The telegram in effect expressed horror at the possibility that JET might not go through and that Europe might not recognise its responsibilities; if JET did not go ahead, it inquired, how many of the team would consider working on the PFTR project at Princeton?

Dr Owen's appeal to the JET team to stay on at Culham, which came after the meeting, was probably made with a eye both to this and to the start of the next school year. Some of the team are thought to have already begun arranging schooling for their children in their own countries, not wishing to wait any longer for a 'final' decision. Their contracts have been extended once again, however, this time to the end of September.

By that time the Foreign ministers ought, with luck and the political will, to have decided on a site. Another meeting is set for 20 September. Between now and then Simonet is expected to visit both London and Bonn to try to iron out the difficulties. His close and long standing relationship with the present Energy and Research Commissioner, Guido Brunner, is expected to help, and though a date has yet to be set, it is on the cards that the British and German heads of government and foreign ministers will meet in Germany for one of their regular bilateral gatherings, the last of which was in London earlier this year.

All will be seeking a way to reach full agreement; a mere majority vote is inadequate under the Community scheme which allows issues not to be decided whenever any single country believes that its national interest is involved. If agreement fails yet again to materialise, JET in its existing form will almost certainly die and Britain and France will probably start initiating their own respective schemes for some other sort of project on a bilateral or trilateral basis. The British prime minister warned of this possibility after the heads of government meeting in June, and there has since been talk in similar terms in both France and Germany.

For the moment the presumption remains that they still wish to cooperate with each other, and to do so through to the Community. It is a presumption increasingly undermined by experience. □

Belgian Embassy

Henri Simonet

EUROPE

Nuclear reports

THE OPPOSITION which the thousands of demonstrators faced at the weekend in expressing their opposition to the Super Phénix fast breeder reactor near Lyons is not confined to the French government alone. The European Commission of the EEC last week voiced its own support for developing fast breeder technology when it released the final two parts of its three-part review on nuclear policy.

Last week's papers were on fast breeders and radioactive waste. A fortnight earlier the Commission released its first paper, on reprocessing. That report, acknowledging the Community's need for nuclear power given its desire to reduce dependence on imported energy, argued that reprocessing would help contain uranium imports and saw greater danger in not reprocessing. The Commission also proposed a special committee to consider a strategy for joint reprocessing.

Last week's breeder report was equally bullish. The limited contribution from new energy sources and the Community's limited access to uranium resources made the breeder an "essential link" in the Community's strategy. Member states had to preserve the option of making the breeder available to utilities on a commercial basis during the early 1990s.

Presenting the paper, Herr Guido Brunner, the Energy and Research Commissioner, insisted that there was no evidence to show that breeders were inherently more dangerous than existing types of reactor, although there were special security problems with plutonium.

- The OECD's Nuclear Energy Agency (NEA), in its annual report published last week, says that even under the most favourable circumstances of high availability and low demand for reprocessing services, the cumulative backlog of unreprocessed material in member countries will increase from 4,000 tonnes U in 1977 to 16,000 tonnes U in 1986, and will remain at that level until 1990. "Uncertainties at the policy-making level" are the main obstacle to adequate reprocessing capacity, it says.

The report also says there is a pressing need to construct large scale demonstration facilities for the solidification and storage of high level waste.

Chris Sherwell

USA

Lost pre-eminence?

The US Office of Technology Assessment has published a report on research in agricultural science. Colin Norman reports from Washington

FOUR major reports in as many years have concluded that basic research in agricultural science in the United States has suffered from financial neglect, and that a modest injection of cash could pay handsome dividends. The latest, a slim volume issued last month by the Office of Technology Assessment (OTA), even goes so far as to suggest that the United States has lost its pre-eminence in basic research to increase food production, because of inflation.

The study, conducted by OTA staff with the help of two outside advisory committees, singles out three areas which should be given particular emphasis—photosynthesis, nitrogen fixation and tissue culture—and it suggests that new funding arrangements should be instituted to ensure that the cash is not siphoned off into other research.

Agricultural research in the United States is mostly funded by the Department of Agriculture, which operates its own research laboratories and channels funds to the State Agricultural Experiment Stations in the form of block grants. No funds are specifically set aside for basic research and, unlike other research agencies in the federal government it does not provide grants for university research on a competitive basis. Virtually every recent study of the agricultural research system in the United States has recommended that the department should institute a competitive grants programme, supporting projects on the basis of peer review; the OTA report is no exception.

One problem with the present system, according to the OTA study, is that since no specific funds are earmarked for basic research, a growing share of the research budget has been taken up by work on immediate problems, such as pest control and disease control, and "the net effect has been to short change basic research whose payoff is long term". Moreover, while basic research has been getting a smaller share of the pie, the pie itself has been shrinking. According to figures compiled by OTA, the Department of Agriculture's total R&D budget this year—$570.6 million —is some $48 million below the 1966 level in terms of purchasing power.

OTA therefore suggests (like all OTA reports, it doesn't actually come out with a hard recommendation) that a competitive grants programme in the Department of Agriculture, specifically designed to boost funding for research on photosynthesis, nitrogen fixation and tissue culture, would pay dividends. Some $15.6 million is now being spent in those three areas by the department, the National Science Foundation, other government agencies and private sources, the report states. It suggests that an additional $12.5 million "appears cost beneficial" in the first year of the new grants programme, and $4–6 million should be added each year for the next six years.

The recommendation is relatively modest. An Academy committee suggested recently that a competitive grants programme should be established with a $60 million initial fund, rising by at least 10% a year in real terms. The programme suggested by the Academy would, however, encompass a vast range of research areas, including studies on nutrition and social policies.

There is at least some indication that repeated calls for such a programme are beginning to be heard in the federal government and in Congress. Earlier this year, President Ford recommended, as part of his valedictory budget, that $27.6 million should be spent on a competitive grants programme in the Department of Agriculture, for a broad range of studies. The funds, in fact, were recommended by the newly-established Office of Science and Technology Policy (OSTP).

A massive agriculture policy bill, now under consideration in Congress, would also elevate research within the department's bureaucratic structure and formally establish a competitive grants programme. The bill has already cleared the Senate, and it was under debate last week in the House.

It is the appropriations committees in Congress which really count, however. Earlier this year, the House appropriations subcommittee which handles the budget for the Department of Agriculture approved only $10 million for competitive grants in the 1978 fiscal year, $17.6 million less than the Administration had recommended. After a bit of lobbying from the White House and from some agricultural scientists, the Senate appropriations committee approved the full $26.7 million, however, and the conference committee which reconciled differences in the House and Senate versions last week came up with a compromise of $15 million. □

USA

- Following the release of a report by the National Academy of Sciences warning of potential long-term climate changes from increased burning of fossil fuels, the Energy Research and Development Administration (ERDA) last week announced the establishment of a new office to plan and conduct a research programme on the problem.

The office will have an initial budget of $1 million, a sum added to ERDA's budget request by President Carter as part of the energy policy he unveiled last April. It will also act as the coordinating unit for other government agencies, and it will keep an eye on international research efforts.

ERDA has also established a panel, under the chairmanship of Dr Alvin Weinberg, of the Institute for Energy Analysis, to assess the potential climatic impact of increased use of fossil fuels, and to recommend a research programme. The panel is expected to produce a report in the next few weeks, and it will form the basis for the new office's research programme.

The Academy committee warned that if use of fossil fuels continues to grow at recent rates, with the industrialised countries switching from oil and gas to coal, carbon dioxide concentration in the atmosphere will probably double over the next century and increase by between 4 and 8 times over the next 200 years. The effect, the committee suggested, may be an increase in average global temperature of about 6 °C toward the end of the twenty-second century.

Though ERDA is taking such predictions seriously, as well it might since the Carter Administration's energy policy calls for sharp increases in the use of coal, ERDA officials are busy noting that there is no immediate crisis and there is ample time to switch to alternative sources of energy if the carbon dioxide problem proves to be serious.

In recent testimony before a Congressional committee, for example, Dr James Liverman, Assistant ERDA Administrator for Environment and Safety, noted that "it is clear that the scientific community cannot today offer the energy strategists a sufficiently confident estimate of the carbon dioxide impact to warrant massive changes in energy forecasting". He added, however, "we must begin discussions with the rest of the world to provide for some flexibility in long-range global energy plans, enough to permit massive reorientation, should it prove necessary, in the next 5 or 10 years".

- Congress last week finally reached agreement on the budgets for the two key basic research agencies in the United States, the National Science Foundation (NSF) and the National Institutes of Health (NIH). Both will get a substantial increase in funds for fiscal year 1978 (which begins on 1 October), though in the case of NSF, the boost will not be as large as the Administration had requested.

In the past few years, NSF's budget request has attracted considerable attention in Congress, as critics of the agency have sought to reduce funds for some of its programmes and to place constraints on its support of school science projects. This year, however, passage was relatively smooth, and NSF officials say they are pleased with the outcome.

NSF will receive $861.3 million next year, a boost of $85.4 million from this year's budget. The Administration had sought $889 million, however, arguing that a major increase is required to offset inflationary losses incurred in support for basic research over the past decade. That argument was challenged strongly by the House Appropriations committee, which stated that "the growth of federal support of academic research has exceeded that of the gross national product by a comfortable margin and has more than compensated for the effects of inflation at a time when other important federal programs were not so favoured". The new budget will, nevertheless, keep NSF a step ahead of inflation.

The only NSF programme to feel Congress's knife strongly this year was the programme of Research Applied to National Needs (RANN), an effort to focus research efforts on pressing national problems. RANN has long been controversial since it puts NSF firmly into the business of supporting applied, rather than basic, research. Congress decreed that RANN should get only $63 million next year, $15 million less than the Administration requested.

As for NIH, a compromise has been reached on the budget bill for the Department of Health, Education and Welfare (HEW), of which NIH is a part, which will significantly increase the Administration's budget request for every institute. The National Cancer Institute (NCI) will again carry off the lion's share of the increases, receiving a budget of $867 million. The National Heart, Lung and Blood Institute comes a distant second with $446 million. The other nine institutes will mostly receive an amount sufficient to account for inflation.

Though the amount budgeted for NIH is unlikely to be changed, the HEW budget bill nevertheless still faces some debate in both the House and Senate on provisions relating to abortion.

- President Carter last week named Arthur C. Upton, Professor of Pathology at the University of New York at Stony Brook, to be the new Director of the National Cancer Institute. The announcement, which had long been anticipated, culminates a long and intensive search for a suitable candidate.

Upton, 54 years old, is an expert on radiation injury and cancer. He recently chaired a committee which recommended termination of a controversial demonstration project, sponsored by NCI and the American Cancer Society, which involved routine breast X-rays for women under the age of 50.

The position of Director of NCI has been vacant since last September, when former Director Frank Rauscher resigned to take a $75,000 per year job with the American Cancer Society. President Ford was reportedly set to nominate pathologist Arnold Brown of the Mayo Clinic for the job late last year, but since he was then a lame-duck president, he decided to leave the appointment to his successor. A search committee was set up earlier this year to look for suitable candidates for the post, and in April it submitted three names —Brown, Upton and Baruj Benacerraf of Harvard.

Colin Norman

CANADA

● Atomic Energy of Canada Limited (AECL) is in trouble again. If last winter was the season of its discontent, it is hard to find a suitable description of its status this summer. Early in July, Energy Minister Alastair Gillespie announced that the Crown corporation had lost about $130 million over a sale of a CANDU reactor to Argentina, and that he had fired the corporation's president, John Foster, as a result. Opposition members called for Gillespie's own resignation because they had warned last winter the loss would be more than $100 mililon, but Gillespie had then maintained it would only be between $23 million and $40 million.

Gillespie rejected their criticism. He did so on the ground that he had invited Ross Campbell to join the board of directors of the corporation, to act as chairman of AECL, and to renegotiate the AECL contract with Argentina. Campbell was a former ambassador to Japan. Gillespie told the House of Commons earlier this year that the renegotiation would reduce the losses, estimated by an auditor-general's report to amount to $100 million because of inflation, by about $60 million to $80 million. The renegotiation was to be made on the basis of the sale of a second reactor.

But Campbell's appointment as acting president of AECL temporarily to replace John Foster brought more criticism. The New Democratic Party's energy critic, T. C. Douglas, asked incredulously in the House: "Do I understand the minister is telling me that prior to the signing of a renegotiated agreement he was given the information that the loss would be cut to somewhere between $23 million and $40 million, that he now finds that those who drafted the agreement and signed it have incurred a loss of $130 million, and that the man who carried on the negotiations now is being appointed president of AECL?"

Gillespie should resign, said Douglas. So did Allan Lawrence, of the Progressive Conservative Party. But Gillespie defended the earlier figures on the loss, saying it was the best information the company had at the time. He defended Campbell by saying that a team of negotiators was involved in the Argentina talks, and that Foster, not Campbell, was then the corporation's chief executive officer.

The government has repeatedly found itself in difficulty over the Argentina sale. At first it was opposed on the ground that it could lead to nuclear weapons proliferation, because Argentina was regarded as unstable politically. Then the House of Commons' Public Accounts Committee questioned a $2.4 million commission paid by Canada for AECL's sale of the reactor to Argentina. The commission was paid into a Swiss bank account held by a trading firm in Leichtenstein, but AECL did not know the name of the agent or whether it was the Liechtenstein firm.

● Another summer landmark has been the passing by the House of Commons of the bill re-organising the federal fund-granting agencies for university research, Bill C–26. Changes in the granting councils were recommended years ago by the Lamontagne committee, the Senate special committee on science policy. The government promised them as long ago as 1974.

Under the new legislation two new fund-granting councils will be created —the Natural Sciences and Engineering Research Council and the Social Science and Humanities Research Council. The former will take over the granting function of the National Research Council, leaving it free to concentrate on its other functions. The latter will take over responsibility for awards in the social sciences and humanities, and the Canada Council, which formerly handled such grants, will deal solely with the arts.

A new Inter-Council Coordinating Committee will maintain liaison and communications between the three award-granting bodies (the third being the Medical Research Council, whose function remains largely unchanged). The new committee, made up of the three council presidents, will be chaired by the secretary of the Ministry of State for Science and Technology.

In discussion in the House, the Minister of State for Science and Technology, Mr Hugh Faulkner, said he believed the re-organisation of the granting councils "will open the way to a new relationship between the government and universities in research matters". He noted that, in the past, there has been "little, if any, coordination of university research objectives regionally or nationally."

Curiosity-oriented research must continue to receive support, said Mr Faulkner, but a more selective process is needed to allow the relatively few individuals whose research is of recognised quality to be supported well—perhaps better than in the past.

The *laissez faire* attitude to the support of university research had a much narrower validity than it once had. This meant the time had come for a "more activist" approach by the granting councils.

● The chairman of the Science Council of Canada, Josef Kates, used the publication of the council's eleventh annual report as an occasion to punch home a theme that has become perhaps the organisation's most important: technological sovereignty.

In his annual statement in the report, Dr Kates said that if Canada was to create long-term stability and respond to the dictates of Canadian geography in an increasingly interdependent world, it must stimulate and control the development of technologies essential to the well-being of the nation. "In this last quarter of the twentieth century, Canada will need to assert *technological sovereignty, that is, to develop and control the technological capability to support national sovereignty,*" he said (his emphasis).

Because of historical factors and recent policies, Canada found itself in a weak position industrially, Dr Kates said. Industries that had been the leading edges of the industrialised economies for the last twenty years were the weakest in Canada. To attain technological sovereignty, Canadians would have to control technologies considered vital to Canada; develop indigenous technological capabilities in selected areas (with government adopting a "buy Canada" policy); encourage internationally-competitive high technology firms in specific areas and develop small, innovative technical firms; and set up major government-sponsored programmes to meet national needs.

At a press conference releasing the report, John Shepherd, executive director of the council, said the first step required in getting major programmes into operation (long a council objective), was to get Cabinet agreement. He referred to the history of oceans systems as a classic example of what happens to major programmes in Canada: Council identified ocean systems as a priority area in 1971, and Madame Jeanne Sauvé, then Minister of State for Science and Technology, approved the idea in a policy statement in 1973. But to date, the government has set no priorities for the programme.

David Spurgeon

IN BRIEF

Séveso row

When the release of dioxin at Séveso last year was considered at an ecotoxicology workshop held this week under the auspices of NATO's Ecosciences panel, the means of assessing the extent of dioxin poisoning in humans generated heated debate. Dr Guiseppi Reggiani, director of medical research for Hoffmann-La Roche, claimed that chloracne, the disfiguring skin disease observed in some 100 Séveso schoolchildren, was an "obligate sign of dioxin contamination".

Professor Silvio Garattini and Dr Gianni Tognoni of the Mario Negri Institute, Milan, the two main Italian participants, delivered scathing replies. Garattini described Reggiani's statement as "dangerous", and insisted that there was "no acceptable scientific evidence" to support it. He couldn't accept the implication that the surveillance programme of all residents, and not just children, was a waste of time. Reggiani adhered to his view, and for the future forecast that there would be "no severe health damage to the population at Séveso". The Italians remained unconvinced.

Lead in water: report

The UK Department of the Environment last week published detailed results of a survey carried out in 1975 on levels of lead in Britain's drinking water. The main findings showed that approximately 4% of houses in Britain received water containing more than 0.1 mg of lead per litre during the daytime. About 9% exceeded this level first thing in the morning after water had been standing in pipes during the night. Less than 1% regularly exceeded 0.3 mg per litre.

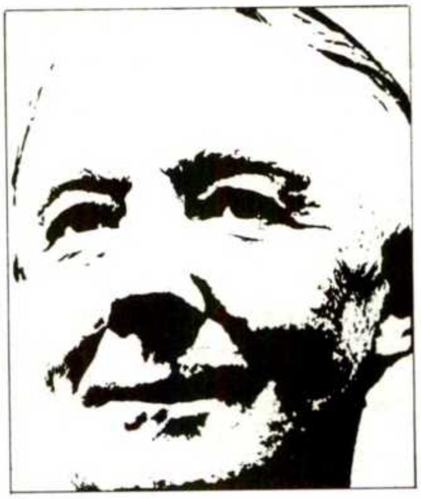
The survey found that water fed into the public supply in Britain contains minimal levels of lead; but in some areas it has a tendency to dissolve lead in pipes. In a statement, the minister, Mr Peter Shore, said there was a need for more research into the precise causes of lead in water, possible ways of minimising it and the effect it has on health.

The World Health Organisation's upper limit for lead concentrations in water is 0.3 mg per litre.

Oil spill policy

The government set out its policy for coping with oil spills at sea last week. Wherever possible, it states, oil spills should be left to degrade naturally; but where there is a serious pollution threat chemical oil dispersants should be used. Provision will be made to cope with spills of 16,000 tonnes per day offshore and 1,000 tonnes per day onshore and in inshore waters.

People projections



KENNETH MELLANBY

For the last ten years we have been bombarded with propaganda about impending world overpopulation. Even in countries like Britain, where the increase is relatively slow, it has been pointed out that if this continues we too will have 'standing room only' within a few hundred years. Though this will not happen, things could become more uncomfortable. But planning even for shorter periods such as the next twenty or thirty years could be much more effective if we had reasonably accurate population forecasts. Our Office of Population Censuses and Surveys (OPSC) makes a valiant effort, but their results have, so far, not been very encouraging.

During the first twenty years after the 1939–45 War, the birth rate was unexpectedly high, particularly among the middle classes, when Britain's 'top people' proudly announced in the *Times* newspaper that they had produced "Jemima, a sister for Jason, Emma, Susan, Giles, Jeremy and Natasha". The country had a minor population explosion. Then during the last ten years the forecasts have been bedevilled by the unexpected and catastrophic drop in the birthrate, so that Britain's population is actually falling.

The effects of this recent change are well illustrated in the recent OPCS Monitor giving population projections for England and Wales up to the year 2010. In 1970 there were about 800,000 live births; the estimate for the total population for 2010 was 62 million. By 1976 births had fallen by some 200,000, and based on this figure the forecast for 2010 was only 51 million. It will thus be seen that, using the methods now available, very small changes in the birthrate have substantial effects on further population projections. Every baby not born this year will subtract something like 50 individuals from the figure for 2010.

In less developed countries, population increase results mainly from the falling death rate. This is less important in Britain, where people mostly live longer, but the OPCS suggest that over the next 40 years there may be a further fall of 10% in the death rate, mainly affecting the young (infant and neonatal mortality) and the aged. This would produce a larger number of men and women over 70, and impose an increasing burden on health services and pension funds. But will this forecast be any more accurate than that about births? We know that rats underfed in the their youth live much longer than their littermates who receive a generous diet. Are overfed babies, toddlers, teenagers and adults likely to survive as well as previous, more austerely raised, generations?

When Britain's population was growing rapidly, in the late 1960s and early 1970s, there were many calls to our various governments to implement population policies to produce, as soon as possible, stability ('zero population growth'). Both Labour and Conservative governments made sympathetic noises, they set up commissions and committees, and they did absolutely nothing.

Britain's politicians must now be kicking themselves for not being able to take the credit for the greatly reduced birthrate. If only in 1969 or 1972 they had promulgated a definite policy—any sort of policy—and had appeared to act vigorously, they could now point out that although they had not stimulated economic growth, built more houses or cured unemployment, their population policy had been completely successful. However, the cynic may say that, had government encouraged population stability, we would all have responded by breeding like rabbits.

correspondence

Translating Chinese texts

SIR,—Whilst preparing a written translation of taxonomic material from Chinese into English some time ago, I was struck by the great disparity between the highly specialised vocabulary of the subject and the simple nature of the grammar employed. This led me to wonder whether Chinese character data-handling techniques, with which I was then involved, might be applied to taxonomic texts to transform them relatively directly by machine into pidgin-English, adequate to convey the original data to the English-speaking reader, working in the appropriate scientific field. This approach seemed viable because Chinese in its written form is morphemic, each character conveying a quantum of meaning, and pidgin, which is itself primarily a direct transformation of Chinese sentences into English on a word-for-word basis, is a basic means of communication over parts of the Pacific Ocean.

An early stage of experiment, using a flowchart which was followed manually, showed that the original idea was promising, but the time-consuming nature of the work meant that relatively little material was processed. Later experiment, however, has involved actual machine processing of a number of texts, and the original ideas have been refined to accommodate some idiosyncrasies of Chinese word order, and to allow smoothing of the finished product. However, these improvements have been achieved without the need to use manual pre- or post-editing, the aim always having been to produce intelligible output by a routine operation which can be run by an operator whose only skill need be the identification of the Chinese characters in the original text.

The process which has been developed is not a linguistically-based general translation programme. It relies for its effectiveness on the careful selection of multi-purpose glosses to represent multi-functional Chinese units in such a way that the reader's general knowledge of the field forces him into the correct choice of gloss for the context facing him. It is only in restricted areas, such as that found in taxonomic texts, that successful transformation can operate, and then it will only produce pidgin, however smoothed, rather than everyday English. However, I feel that the results so far indicate probable eventual success in providing a workable first sieve for the assessment of the large bulk of Chinese taxonomic data which at present seems to be largely unavailable to the Western scientist.

In spite of its inherent interest, it would be of little practical use to develop pidgin transformation further in the absence of any live demand for such a facility. If any readers feel that this type of transformation of taxonomic material would be of benefit to them, I would be grateful to hear from them. To give some idea of the form of output from the process, I append the result of one machine transformation, which shows the general style, and also illustrates how the multi-choice glosses operate (see below).

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Trinucleus Murchison 1839.

     glabella(e/r) all/both(have) convex (front) lobe(s), (has/have)
three pair(s) short, deep glabella(e/r) lateral furrow(s), rear two
pair(s) relatively deep relatively long;

fringe(s) upper lamella(e)'s small pit(s), irrespective of whether at/on
anterior portion and/or lateral portion(s) equally deep, distributed
within the radial sulcus/i.

     pygidium/al broad, breadth approximately is length's 3 cr 4 times.

     genotype: Trinucleus fimbriatus Murchison.

     Period distributed: Asia and(also) Europe                     early
and(also) middle Ordovician.
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Wind power for the UK

SIR,—Some comments are necessary on Martin Ryle's article on 'Economics of alternative energy sources', (12 May, page 111). Whilst his suggestions on storage systems may be useful, his claim that wind power could be a substantial energy resource without much environmental impact is grossly exaggerated.

The coastal area of the UK is not 1.5×10^4 km^2 as stated in the article; in fact the total land area of the UK[1] is only 2.4×10^5 km^2. To obtain an annual output of 0.56×10^9 GJ at a mean density of 2.8 GJ m^{-2} from a swept area that is 4×10^{-3} of the ground area (all figures quoted), one would need an area of 5×10^4 km^2. Thus a fifth of the total area of the UK would have to be covered with towers about 60 m in height at distances apart of about 1 km. It is questionable whether this is practicable. The costs of siting the aerogenerators over all types of country have not been estimated.

In any case the visual impact on the environment would be overwhelming, and in our windier more scenic areas the view would mainly consist of endless rows of towers stretching in every direction. Also the question of noise from the 1 MW turbines, which will be only 1 km apart, is not mentioned.

A more practical upper limit on energy from wind power has been estimated by the Energy Technology Support Unit at Harwell to be the equivalent of about 8 million tons of coal a year from 10^4 generators each of about 1 MW rating[2]. Without an immense cost to the environment wind power could not make a substantial contribubtion to Britain's energy requirement.

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[1] The New Oxford Atlas (Oxford University Press, 1975)

[2] Energy R&D in the United Kingdom. A Discussion Document (1976).

news and views

Experimenting with controlled gravity

from P. C. W. Davies

Our everyday experience of gravity is so pronounced that it is easy to forget what an incredibly weak force it really is. Only our proximity to a mass as large as the Earth enables terrestrial gravitational forces to compete with other forces of nature. By contrast, in the laboratory, gravity is almost completely undetectable: the force of attraction between two eggs in contact is a puny 10^{-6} dyne. This state of affairs has led to an extreme paucity of actual experiments in gravitational physics, because most of them have to rely on the arrangement of astronomical masses in the Solar System to produce effects which are sufficiently strong to be detectable. Unfortunately, the limited repertoire of planetary activity prevents some features of gravity from being measured at all. There have been two major theories of gravity: Newton's and Einstein's. Both have relied heavily on astronomical effects in the Solar System for their verification.

Because gravity is so ill-investigated, almost any proposal for a new experiment is welcome. In a paper in this month's *Physical Review*, three physicists, V. B. Braginsky of the Moscow State University, C. M. Caves and K. S. Thorne of Caltech (*Phys. Rev. D*. **15**, 2047; 1977), propose a collection of novel experimental techniques for measuring gravitational effects, some of which have never been observed but are predicted by theory. The essence of the new proposals is that rapid advances in technology are opening up the prospect of detecting in the laboratory sources of gravity which are under the control of the experimenter. None of the experiments discussed is currently feasible, but several could become so within a decade with sufficient research and development. Some of the technology gained could even have useful spin-off in other fields.

Laboratory-generated gravitational forces are so tiny that equipment of immense sensitivity is needed to detect it. Three new low-dissipation sensing systems are considered by the authors: a torque balance made of quartz or sapphire fibres cooled to 0.1 K, massive dielectric monocrystals cooled to millidegrees, and microwave resonators with superconducting walls. Typically, the source of the fields would be a mass of about 100 kg rotating about 1,000 times a second, which would be capable of producing accelerations due to higher order (post-Newtonian) effects in its vicinity of about 10^{-17} cm s^{-2} (or 10^{-20} g). The major technological problem is how to isolate this effect of interest from other environmental disturbances, particularly seismic effects, which are many orders of magnitude larger. One answer is to conduct the experiments in Earth-orbiting satellites; less expensively, some of them could be performed in tandem and the seismic perturbations subtracted, or they could utilise high frequency time-dependent fields, far above natural seismic frequencies.

Some of the experiments are reminiscent of nineteenth century electrodynamics. One involves the analogue of Ampère's experiment, which demonstrated magnetic forces between current-carrying wires, only in this case the role of the electric current is taken by rotating masses one of which is part of a torsion balance. The rotation period of the other mass is modulated at the natural frequency of the balance in an attempt to set up torsional deflections. A corotating arrangement causes repulsion, and counter-rotation attraction. This 'magnetic' gravity force is expected on theoretical grounds, but has never been observed. Even in ideal laboratory conditions, using light but tough ceramic masses, the problems of untangling the minute 'magnetic' effects from the already tiny, but much larger Newtonian gravitational attraction, not to mention the disturbances from seismic vibrations, are daunting. Nevertheless, the authors do not think it beyond the ingenuity and technological expertise of the next 'generation' of experimenters.

Other possible experiments using the torque-balance are a check on any time-dependence in the strength of gravity (which is predicted by some theories) and a measurement of the gravity produced by magnetic stresses. In the former set-up the entire apparatus, consisting of two massive balls straddling the balance, is made out of monocrystal sapphire cooled to 2 K and kept in a constant temperature environment to eliminate thermal fluctuation noise. After one month, a limit on variation of the Newtonian constant G of about one part in 10^{11} yr^{-1} could be achieved, which is already comparable to astronomical techniques. Turning to the latter experiment, it is particularly important for theory, because general relativity predicts that not only mass, but stress, contributes to gravity. Indeed, in some astrophysical situations, stress-induced gravitational fields can be extremely important, so a direct measurement of the gravity of stress would be of great significance.

In another class of experiment the authors discuss the use of a toroidal cavity filled with a standing-wave pattern of electromagnetic radiation. If this resonator is placed at rest round a rotating mass, according to the theory of relativity the wave pattern should be dragged around in the direction of rotation, an effect known as the dragging of inertial frames. For a mass of 5,000 kg rotating with an equatorial speed of 1 km s^{-1}, the dragging effect works out at a mere 6×10^{-21} radian s^{-1}, which is small by any account. Nevertheless if future technology enables the construction of a resonator having superconducting reflecting walls with a damping time (against decay of the wave pattern) of 10^5 s—which is difficult but not outrageous—then deflections in the wave pattern of 3×10^{-16} radians are feasible, which might be detectable over a duration of about 2 weeks. An improvement in the

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reflection coefficient R of the cavity walls, from the presently attainable $1-10^{-11}$, to $1-10^{-14}$ would be necessary to sustain an experiment of this duration.

The third class of experiment utilises massive dielectric monocrystals in which the experimental arrangement is such as to eliminate the ordinary Newtonian force, which usually dominates all else, and to detect just the post-Newtonian subtle correction effects of interest. One of these experiments is the analogue of the Faraday effect in electrodynamics, in which a changing magnetic field threading a detector coil induces an electromotive force (the principle of the transformer). In the gravitational variant, the 'magnetic' induction is produced by a uniformly rotating vertical cylinder set coaxially with an axisymmetric, specially shaped, sapphire crystal, which is free to perform torsional oscillations. As the cylinder is moved up and down at the natural frequency of these oscillations, changing 'gravimagnetic' flux linking the two masses drives the torsional oscillations The force involved is incredibly small—producing accelerations of only 10^{-18} cm s^{-2}. In this regime even the act of measurement of the oscillations could introduce Newtonian gravitational forces between the masses which are of comparable strength.

Less feasible experiments are also discussed in which gravitational fields are detected which originate, at one extreme with the uneven distribution of matter in the Galaxy, and at the other with a beam of relativistic protons from a storage ring. In the latter experiment, a pulse of high velocity protons passes close to a monocrystal detector at periodic intervals in an attempt to set up resonant oscillations through gravitational coupling. If successful, the experiment would discriminate between scalar, vector and tensor gravitational fields, and is hence of considerable theoretical interest. Technological problems abound though, and little investigation of disturbing effects, such as bombardment of the detector by radiation from the storage ring, has yet been conducted.

All in all, this analysis provides a speculative but thought-provoking basis for more detailed feasibility studies. Much of the current impetus in experimental gravity research is behind astronomical and spaceflight technology. With the colossal cost involved in conducting extraterrestrial experiments, perhaps the time has come for a more penetrating appraisal of the possibilities for detecting and measuring laboratory-manipulated gravity. ☐

Reading the tumour virus genome

from B. W. J. Mahy

THE strategem by which an RNA virus directs the host cell to synthesise virus-specific protein components continues to fascinate biochemists. Broadly, the RNA viruses are comprised of two groups, those in which the genome RNA acts as messenger for protein synthesis (positive-strand viruses) and those in which the genome RNA is of the opposite sense to messenger, and must be transcribed into a complementary RNA species (cRNA) to initiate multiplication (negative-strand viruses). The RNA tumour viruses (oncornaviruses) along with picornaviruses (such as polio), togaviruses (such as Sindbis) and most plant viruses, fall into the first group. Thus genome RNA extracted from these viruses is an active messenger in cell-free protein synthesising systems and, in the case of picornaviruses, RNA of genome size is found on polysomes of infected cells where it translates into a full-length polyprotein that is subsequently cleaved into the capsid and other virus-specific proteins.

But in many other cases the situation is not so simple. The 42S genome RNA of togaviruses is infectious and can direct the synthesis of some polypeptides in a cell-free system but these are not the virus structural polypeptides. A subgenomic species of RNA (26S) found on infected cell polysomes is the messenger for the synthesis of a polyprotein which undergoes post-translational cleavage to form the virus structural proteins (Cancedda *et al. J. Virol.* **14**, 652; 1974; Clegg & Kennedy *FEBS Lett.* **42**, 327; 1974; Simmons & Strauss *J. molec. Biol.* **86**, 397; 1974; Wengler *et al. Virology* **61**, 120; 1974). This 26S RNA represents one-third of the genome and is located inward from the 3′ end of the 42S RNA (Kennedy *J. molec. Biol.* **108**, 491; 1976). Translation of the 42S RNA itself is initiated at a single site near the 5′ end (Cancedda *et al. Cell* **6**, 215; 1975) but terminates before the structural protein genes; the product is a polyprotein which is cleaved to form various non-structural polypeptides, probably including components of the viral replicase (Brzeski & Kennedy *J. Virol,* **23**, 420; 1977). It seems that a similar type of translational control is employed during the replication of some plant viruses. Tobacco mosaic virus RNA, for example, directs the synthesis *in vitro* of two non-structural polypeptides which are components of the viral replicase, but the coat protein gene located near the 3′ end is not translated. The functional mRNA for coat protein is a subgenomic species found in infected cells (Hunter *et al. Nature* **260**, 759; 1976).

Currently, interest in the translation of the RNA tumour virus genome is at fever pitch. These viruses replicate by transcription of a DNA provirus integrated into the host cell genome, and virus-specific polysomal RNA is of the same sense as the genome RNA. The reason for the current excitement is the clear demonstration from several groups that the genome RNA of viruses which induce sarcomagenic cell transformation (non-defective viruses) contain a distinct gene (*src*) located near the 3′ end (Joho *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 4772; 1975; Wang *et al. J. Virol.* **16**, 1051; 1975; Stehelin *et al. J. molec. Biol.* **101**, 349; 1976). Identification of the *src* gene product is crucial to our understanding of the mechanism of cell transformation. Since transformation-defective viruses lack this gene, comparison of the *in vitro* translation products of their RNA with non-defective virus RNA should yield information on the nature of the *src* polypeptide.

The genome of RNA tumour viruses consists of two identical 35S subunits each of molecular weight $3{\cdot}3\times10^6$ and containing genes for internal structural proteins (gs antigens, *gag* gene) envelope glycoproteins (*env* gene) and the virus RNA-dependent DNA polymerase (*pol* gene) in addition to *src*. The genes of avian oncornaviruses have been mapped in the order 5′-*gag*-*pol*-*env*-*src*-3′ (Wang *et al. Proc. natn. Acad. Sci. U.S.A.*, **73**, 1073, 1976), and it is highly probable that the same gene order occurs in the murine oncornavirus genome.

Recently Alan Smith's group at Imperial Cancer Research Fund translated the genome RNA of Rous sarcoma virus in a variety of cell-free protein-synthesising systems (Pawson *et al. J. Virol.* **19**, 950; 1976). In each case the product turned out to be a polypeptide of molecular weight 76,000 which was related to the internal structural proteins (*gag* gene) as judged by immunoprecipitation and peptide mapping. The other virus gene products

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were not detected, and no differences were observed between non-defective and transformation-defective virus RNA. Duesberg's group obtained similar results (von der Helm & Duesberg *Proc. natn. Acad, Sci U.S.A.* **72,** 614; 1975).

An important finding by Pawson *et al.* was that the 76,000 molecular weight product could be labelled *in vitro* with chemically formylated ^{35}S-Met-tRNA$_f^{Met}$, which only labels at the N-terminal position. This confirmed that the 76,000 polypeptide was a primary translation product and not derived from cleavage of a larger molecule.

In this issue of *Nature* (page 416) Pawson *et al.* have extended their work by examining the translation products of RNA obtained from Rous sarcoma virus-infected cells. Two size classes of virus-specific RNA, one around 3×10^6 daltons (35S) as in the viron, the other around 1.3×10^6 daltons (24S), are found in the cytoplasm of cells infected with avian sarcoma virus (Bishop *et al.* in *Animal Virology,* ed. Baltimore D. & Huang A. S., Academic Press, 1976). Pawson *et al.* find that the intracellular 35S RNA when added to cell-free protein-synthesising systems codes for only one polypeptide of molecular weight 76,000 which corresponds exactly to the *gag* gene product synthesised from virion RNA. However when they add to the system 20–25S RNA from infected cells, a new polypeptide of molecular weight 70,000 is synthesised, and this is immunoprecipitated only by antiserum against the virus glycoproteins. It seems therefore that the 20–25S from infected cells encodes the *env* gene and is a subgenomic species similar to the 26S RNA of togaviruses.

Results in agreement with these have been obtained by Hanafusa's group at the Rockefeller University, who microinjected either 35S or 21S RNA from avian leukosis virus-infected cells into chick embryo fibroblast cells infected with the Bryan high-titre strain of Rous sarcoma virus, which lacks the ability to synthesise its own envelope glycoproteins. Production of infectious Rous sarcoma virus provided a sensitive assay for envelope mRNA and it was found that 21S RNA but not 35S RNA expressed this activity (Stacey *et al. Proc. natn. Acad. Sci. U.S.A.* **74,** 1614; 1977).

Recently, very similar findings have been made with the murine RNA tumour viruses. Both 35S and subgenomic (20–25S) RNA species are found in cells infected with these viruses (Fan & Baltimore *J. molec. Biol.* **80,** 93; 1973; Gielkens *et al. Proc. natn. Acad. Sci. U.S.A.* **71,** 1093; 1974), but the 35S RNA species alone is found on polysomes immunoprecipitated with antiserum to virus internal structural protein (Mueller-Lantzsch & Fan *Cell* **9,** 579; 1976). Direct translation of the two intracellular RNA species has recently been achieved by injection into *Xenopus* oocytes (Van Zaane *et al. Proc. natn. Acad. Sci. U.S.A.* **74,** 1855; 1977). The products, identified by immunoprecipitation, were shown to be internal structural proteins (*gag* gene) for the 35S RNA and envelope polypeptides (*env* gene) for the 22S RNA.

From these results it seems clear that the only 'open' cistron available for translation from the 35S genome RNA of oncornavirus is the *gag* gene, located nearest to the 5′ end. Presumably the internal initiation sites for the other gene products are blocked in an analogous manner to the structural protein genes of togaviruses. Several problems remain. The *env* mRNA can be identified as a discrete species in infected cells, but the mechanism of its synthesis by post-transcriptional processing or by some other means is not known. It may be relevant that subgenomic negative-strand RNA species which could be templates for mRNA synthesis have recently been identified in cells infected with RNA tumour viruses (Stavnezer *et al. J. Virol.* **20,** 342; 1976).

The mRNAs coding for the *pol* gene product or the *src* gene product have not been identified as yet. However, if the model proposed by Pawson *et al.* is correct, one possible way to get at the *src* gene product might be to use suppressor tRNAs to allow readthrough of all the genes in 35S RNA from transformation-defective and non-defective viruses. □

Amorphous semiconductors

from A. R. Long

The 7th International Conference on Amorphous and Liquid Semiconductors was held at Edinburgh on 27 June–1 July, 1977.

In the past, the study of amorphous semiconductors has been only loosely related to the wider field of study embracing the analogous crystalline materials. It has rather been dominated by ideas of electron localisation peculiar to disordered lattices. However, recent developments reported at the conference imply a significant change of emphasis, with a much stronger concentration on the non-crystalline analogues of well-known phenomena of the ordered state. This development was stimulated by the now well-documented discovery of the technique for doping amorphous silicon films prepared by the glow discharge decomposition of silane, to produce the familiar n-type and p-type materials and p-n junctions.

W. E Spear and his coworkers (University of Dundee) presented a number of papers giving a coherent account of the electronic properties of doped amorphous silicon prepared by their glow discharge technique. Other groups, notably from IBM, Xerox, Harvard and the University of Chicago, working with similar material, emphasised the importance of hydrogen incorporation in these films in reducing the density of defect related electron states within the energy gap and thus enabling doping to take place However, it is not possible to say at present if the substantial proportions of hydrogen contained in these films are themselves influencing the band structure of the material nor whether the doping process *per se* disorders the network further, thereby changing the intrinsic properties of the material as well as moving the fermi level. Much work remains to be done on these materials, particularly as they are of potential commercial importance.

The anomalous sign of the Hall effect observed for some amorphous semiconductors has long been a puzzle. D. Emin (Sandia Laboratories) described the manner in which the sign of the Hall coefficient is influenced by the connectivity of the appropriate structural units in the network and also the progress made in accounting for the magnitude and temperature dependence of the Hall mobility With this work, an important technique in developing our understanding of crystalline semiconductors can now be applied with more confidence to the amorphous state.

The most striking recent theoretical advance in the physics of disordered materials has been the realisation, initially by Street and Mott, that the nature of electronic defect states may be profoundly influenced by the local atomic configuration, and in particular that pairs of singly occupied dangling bond states may in certain circumstances be energetically unstable and tend to form their charged analogues.

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These ideas may be used, as was reported in a number of papers, to explain a wide variety of low temperature photo-induced properties of chalcogenide glasses. Moreover, when interpreted within the chemical framework appropriate for chalcogenides, the 'valence alternation pair' model of Kastner, Adler and Fritzsche, they provide a convincing explanation of why it is not possible to move the fermi level in chalcogenides by doping in the traditional manner. Investigations were reported, by a group from Energy Conversion Devices led by S. R. Ovshinsky, of the modification of chalcogenide transport properties by the introduction of considerable atomic fractions of charged impurities into the matrix. However, the exact nature of the changes occurring in this modification process and the influence of the preparation conditions used are unclear at present, and it remains to be seen whether such modified chalcogenides will be of any lasting importance.

One interesting feature of the conference was the dominating position of certain lines of investigation and the virtual eclipse of topics which have dominated previous meetings. Little new structural work was reported, possibly because of the difficulty in interpreting unambiguously the results from traditional structural probes. However, the relatively new study of X-ray absorption fine structure (EXAFS), reviewed for the conference by D. E. Sayers (North Carolina State University) is an important advance, in that individual atomic species in multicomponent systems can be studied separately, and new results using this method were reported, particularly striking being the investigation of the coordination of arsenic impurities in amorphous silicon by the Xerox group. Recent studies of liquid semiconductors were restricted to two sessions, concentrating on conduction mechanisms. The thermal and vibrational properties of the disordered state which show highly anomalous features, were comparatively neglected, though important new results interpreting the dielectric properties of low temperature glasses in terms of polar two-level atomic states were presented by the Stuttgart/Grenoble group.

Papers concerned with applications of amorphous semiconductors were also few. David Adler (Massachusetts Institute of Technology), in a subtle and convincing analysis of a particular class of chalcogenide threshold switching devices, was able to demonstrate that thermal effects are insignificant and that electronic mechanisms are responsible for initiating and sustaining the on-state. Although there is undoubted interest in the fabrication of thin film photovoltaic cells for energy conversion, little progress was reported beyond a definition of the design criteria, which will have to be met. It will be interesting to see if our enhanced understanding of basic processes can be turned into marketable devices by the time of the next international conference in this series.

From the papers presented at this conference, it is apparent that the study of amorphous semiconductors has progressed beyond the stage where uncertainties in the preparation conditions had a profound influence on observations. If an amorphous material is carefully prepared under well controlled conditions, and suitably characterised, it will in general be possible to make consistent measurements of its physical properties, which was by no means true in the early years of the subject. However, our theoretical understanding of many of the processes taking place in these materials is still weak, and the identification of the specific structural features responsible for particular effects almost nonexistent. It is to be hoped that, with more reliable experimental data becoming available all the time, these deficiencies will soon be rectified. □

Calcium and muscle regulation

from S. J. Smith

MUSCLE contraction involves the repetitive interaction of crossbridges from thick filaments with thin filaments, impelled by the hydrolysis of ATP. Although calcium is the universal intracellular signal which triggers contraction, there seem to be two targets for its action. In vertebrate skeletal and cardiac muscle, a subunit of the troponin complex on the thin filament is the calcium receptor; in the absence of calcium this complex impedes the interaction between actin and myosin. According to the currently favoured 'steric blocking' theory, calcium binding is accompanied by a movement of tropomyosin, a rod-like protein, which lies in the two grooves formed by the double helical array of actin monomers; the myosin-binding sites on actin are thereby exposed, allowing contraction to occur. In contrast, in invertebrates, regulation has been found to be linked to the myosin molecule, involving specific regulatory light chains. The most extensively studied system is that of the scallop, where the selective release of one of the two chemically identical regulatory light chains by EDTA results in the abolition of calcium sensitivity of the actin–myosin interaction (Kendrick Jones *et al.* *J. molec. Biol.* **54,** 313; 1970). This is accompanied by the loss of half of the calcium-binding sites on the parent myosin. Calcium regulation is completely restored by replacement of the absent light chain in the presence of magnesium. This suggests that both light chains are necessary for regulation, and work by Szent-Györgyi *et al.* (*J. molec. Biol.* **74,** 179; 1973) implies that both myosin heads are cooperatively involved.

The advantages of the different types of regulation are not yet understood, and do not seem to be correlated with the speed of contraction of the muscle (see discussion by Lehman *Int. Rev. Cytol.* **44,** 55; 1976). In some phyla both regulatory mechanisms exist. The approach of Harris and Epstein (*Biochemistry* **16,** 859; 1977), who are investigating the contractile proteins of mutants of a 'dual-regulated' nematode worm, may prove valuable if mutants defective in one or other of the regulatory systems can be isolated.

There is no simple phylogenetic pattern for distribution of the regulatory systems. Myosin-linked regulation is not confined to invertebrates, but has been demonstrated in the smooth muscle of higher vertebrates (Bremel *Nature* **252,** 405; 1974). Recently, the mechanism of this regulation has been extensively investigated in chicken gizzard and pig stomach muscles (Sobieszek *Eur. J. Biochem.* **73,** 477; 1977; Small & Sobieszek *Eur. J. Biochem.* **76,** 521; 1977). It seems that the triggering of the actin–myosin interaction is brought about by calcium-dependent phosphorylation of the 20,000 dalton light chain by a specific light chain kinase. This occurs very rapidly and *in vitro* can be shown to precede the rise in actin-activated ATPase activity, suggesting that the process is physiologically important. Dephosphorylation occurs through the action of a phosphatase, which is not calcium sensitive and is present in approximately equivalent amounts to the kinase. The site of action of calcium is not yet certain; although smooth muscle myosin itself binds calcium in significant amounts, the isolated 20,000 dalton light chain does not, although it can be phosphorylated in the presence of calcium. This would suggest that calcium acts solely in the phosphorylation process but could also be consistent with a scheme whereby some interaction with the heavy chain is necessary for calcium binding by the light chain. The part that tropomyosin could play in a myosin-linked regulatory system was unclear. However, recently Sobieszek

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& Small (*J. molec. Biol*, **112**, 577; 1977) have shown that tropomyosin is necessary for amplification of the affect of phosphorylation, being required for the full development of actin-activated ATPase activity. It would seem that, on calcium activation, as in skeletal muscle (reviewed by Weber & Murray *Physiol. Rev.* **53**, 612; 1973), tropomyosin brings about a cooperative change in the actin filament, which facilitates the actin–myosin interaction.

Whereas myosin-linked regulation in smooth muscle now seems to be indisputable, it is not certain whether troponin is present in these muscles. Ebashi reports (Mikawa *et al. J. Biochem.* **81**, 273; 1977) an 80,000 molecular weight calcium-sensitising factor, which has no kinase activity. Moreover troponin C, the calcium-binding component of the complex, has been isolated from uterus muscle by affinity chromatography using troponin 1, the inhibitory unit of the skeletal muscle complex (Head *et al. Biochem. J.* **161**, 465; 1977). However, *in vitro* functional tests suggest that there is no thin filament regulation (Sobieszek & Small *J. molec. Biol.* **102**, 75; 1976). It seems possible that this calcium-sensitising factor performs some other, as yet unrecognised function in smooth muscle. In this context, it is of interest that a troponin C-like protein, which will restore calcium sensitivity to desensitised skeletal actomyosin, has been isolated from brain, fibroblasts and neurosecretory tissue, where it seems to modulate cyclic nucleotide turnover. (Amphlett *et al. FEBS Lett.* **72**, 163; 1976).

In the light of these new findings of calcium-dependent phosphorylation as the trigger for actomyosin ATPase activity in smooth muscle, it seems valuable to consider the possibility of myosin-linked regulation in skeletal muscle. A calcium-sensitive light chain kinase, distinct from that in smooth muscle, has been demonstrated in skeletal muscle, but no link between phosphorylation and actin-activated ATPase activity could be found (Perrie *et al. Biochem. J.* **135**, 151; 1973). There are suggestions that phosphorylation and the calcium response of skeletal myosin may be intimately related (Okamoto & Yagi *J. Biochem.* **80**, 11; 1976). Until recently, there was no *in vitro* evidence for calcium regulation by skeletal myosin. However, Lehman (*Biochem. J.* **163**, 291; 1977) has demonstrated that this can occur in some conditions.

All the evidence so far accumulated indicates the complexities of control mechanisms potentially available for the precise regulation of excitation–contraction coupling; an assessment of their physiological importance will provide a step further towards the seemingly ever-receding mirage of a complete understanding of muscular contraction. ☐

Cosmic ray age, soft X-rays and the galactic wind

from J. J. Quenby

ALTHOUGH the distribution of stars in our Galaxy is in the form of a thin disk, a strong flow of plasma perpendicular to the plane may produce a magnetic halo of near circular shape. Recent cosmic ray balloon measurements carried out by Webber, Lezniak, Kish and Simpson of the University of New Hampshire (*Astrophys. Lett.* **18**, 125; 1977) may provide data which aids the modelling of the phenomena involved.

Initial experimental observation of the presence of a galactic halo which follows the figure of an ellipsoid of revolution come from cosmic radio noise data at hundreds of MHz. Relativistic cosmic ray electrons, spiralling in weak, few microgauss or less magnetic fields, are thought to produce the radio noise by way of the synchrotron radiation process. The halo constitutes a possible magnetic reservoir for cosmic rays and these particles could therefore spend the greater part of their lives well outside the galactic disk.

A recent, dynamical argument for the existence of the halo has been put forward by Ipavich (*Astrophys. J.* **196**, 107; 1975). He starts from the idea that the anisotropic motion of cosmic rays along the interstellar magnetic field lines may generate and amplify magnetohydrodynamical waves by a scattering process, but the waves in turn lose energy to the interstellar gas. The net result is that cosmic rays heat the interstellar medium and a situation, analogous to the solar wind in the solar corona, may arise. An out-of-the galactic plane flow is initiated which eventually becomes supersonic in a manner similar to the sub to supersonic transition at the neck of a rocket motor nozzle. Gravity perpendicular to the disk plays the same part as the constriction in the motor nozzle. Hence interstellar field is carried out by this galactic wind to constitute an additional, low density medium reservoir for the cosmic rays.

Studies of the relative abundances of the cosmic ray isotopes (Li, Be, B) known to be produced by nuclear interactions in the interstellar medium suggest that the mean amount of matter transversed by the cosmic rays is $\sim 5\ \mathrm{g\,cm^{-2}}$. Taking this figure in conjunction with the $\sim 1\ \mathrm{cm^{-3}}$ number density of interstellar atoms implies a cosmic ray life of about 10^6 yr. An alternative and more direct measure of this life lies in finding the amount of radioactive ^{10}Be relative to its decay product ^{10}B existing in the cosmic ray flux. The 1.5×10^6 yr beta decay half life of this process is conveniently matched to the expected cosmic ray age. Spacecraft telescopes flown by Garcia Munoz *et al.* (*Astrophys. J. Lett.* **201**, L141; 1975) detected few or no ^{10}B particles, suggesting a most probable age $\sim 2\times10^7$ yr. These workers reconcile their age measurement with the order of magnitude smaller apparent value given by the Li, Be, B abundances by supposing that cosmic rays spend most of their life in the halo where the matter density may be one hundredth of that in the disk. Unfortunately, conflicting experimental evidence is available from a much larger, but balloon borne, detector flown by Hagen *et al* (*Astrophys. J.* **212**, 262; 1977) which recorded enough ^{10}Be to be consistent with an age of only 5×10^6 yr. Webber and coworkers have now performed a balloon experiment in a similar energy range to that of Hagen *et al.* but at a greater altitude so that fewer unwanted atmospheric interactions take place. Also this latest detector seems to achieve better mass resolution than that of previous balloon experiments. Webber *et al.* carefully consider the distortion effects of solar modulation on the relative ^{10}Be abundance (see *News and Views* **259**, 11; 1976) and find that the mean cosmic ray life must be at least 8×10^6 yr and could be 15×10^6 yr or more. They conclude that their data would allow either a disk or halo model for cosmic ray confinement.

Owens and Jokipii (*Astrophys J.* in the press) have set the halo model for cosmic ray motion within the context of Ipavich's work. They show that a cosmic ray age of more than 10^7 yr requires a halo scale height of 4 to 10 kpc and an average galactic out-of-plane wind velocity of $<60\ \mathrm{km\,s^{-1}}$, numbers consistent with Ipavich's estimates. It is interesting to note that Ipavich also requires an interstellar gas temperature in the 10^4 to 10^6 K range. Soft X-ray background survey data points to a hot galactic gas temperature $\sim 6\times10^5$ K as revealed by rocket measurements made by Levine and coworkers at MIT. Further insight into the dyanmics of a hot galactic gas can arise from a consideration of the X-ray emission from rich clusters of galaxies such as COMA. The soft X-ray line and continuum data on the

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COMA cluster are consistent with a model in which a hot, intercluster gas receives its energy from heating inside the individual active members of the cluster (see Serlemitsos *et al. Astrophys. J. Lett.* **211,** L63; 1977 for experimental data; Yahil *et al Astrophys. J.* **185,** 787; 1973 for theoretical discussion). Cosmic ray heating could well be the relevant non-thermal process feeding the intercluster medium through enhanced galactic winds. ☐

Forecasting in crop protection

from R. Hull and C. R. B. Baker

> The Second Conference on Forecasting in Crop Protection was held in Paris on June 21–24 by the European and Mediterranean Plant Protection Organisation.

THE ability to forecast reliably the timing and intensity of pest and disease attack on crops is crucial to reducing unnecessary and undesirable use of pesticides if at the same time agricultural productivity is to be maintained and improved. This claim was made for many national forecasting schemes described at the conference, which covered national forecasting organisations, meteorology, field monitoring methods for pests and diseases, interpretation of pest and disease counts, development of forecasts, and the link-up with the growers.

Schemes of varying sophistication were outlined. They ranged from a computer-based system organised by Michigan State University in the US, through national networks of observers and warning stations operated in countries such as France and Hungary, to systems designed to provide warnings of a specific pest or disease. Techniques on which forecasts are based include monitoring pests and diseases by sampling crops, trapping insects in pheromone traps and in tall suction traps that sample the migrant fauna in the air, observations on caged pests, observations on plots of appropriate crops maintained at regional forecasting centres and the use of meteorological data. In most countries such schemes are operated by government agencies or are subsidised by government funds. Since the activity of all organisms damaging crops is influenced in one way or another by weather, much attention was given to recording and interpreting meteorological data in relation to phytopathological problems.

Means of communication with farmers and growers included postal warnings, radio and television broadcasts, telephone answering services, public notices and personal contact. Although delegates seemed convinced that their own national schemes were making some impact on farming practice, especially in reducing use of pesticides, little quantitative evidence was presented that pests and diseases were being controlled more efficiently or that the forecasting schemes were economic. It was emphasised that forecasts were generally issued to cover a region and cannot be detailed enough to show the local variations that affect the individual grower. He needs local advice or further education so that he can make his own judgement based on the growth stage of his own crops and the actual stage and abundance of the pests or diseases present and also sometimes of their natural enemies.

Several speakers stressed the need for a thorough knowledge of the biology of pests and diseases, but important gaps in such knowledge were evident throughout the conference. In particular there was a lack of a coherent picture of differences in pest behaviour across Europe. Standardisation of assessment methods as recommended in the FAO manual on Crop Loss Assessment Methods was discussed.

Agricultural research and advisory services have been operating in most developed countries for several decades. It was clear from this conference that despite all the effort applied so far, problems in pest and disease management continue and that forecasting is an essential part of any ultimate solution. Unless flexible, the best devised research and advisory programmes could be nullified by a change in agricultural practice, such as that from ploughing to direct drilling.

The conference made two proposals for follow-up action under the aegis of EPPO. First, a working party should be convened to appraise the various systems of assessing and forecasting crop losses. Second, closer cooperation should be organised between meteorological and plant protection services in the collection, dissemination and use of meteorological observations in relation to the needs of crop protection. ☐

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Tree-ring dating in Britain comes of age

from a Correspondent

> The first International Symposium on Dendrochronology in Northern Europe was held at the National Maritime Museum, Greenwich, London from 11 to 14 July, 1977. The papers will be published by *British Archaeological Reports* (112, Banbury Road, Oxford OX2 7BP) in collaboration with the National Maritime Museum and the Research Laboratory for Archaeology and the History of Art, University of Oxford. A companion exhibition, instigated by Dr J. M. Fletcher (University of Oxford), on tree-ring analysis of Tudor portraits is open at the National Portrait Gallery, London until 18 September 1977.

IN the historic setting of the National Maritime Museum close by the Royal Observatory, Greenwich Park—long famous for the measurement of British Standard Time—the precise measurement of the annual growth rings of trees for dating purposes was shown at the meeting to be an interdisciplinary and international subject with great potential for European environmental science and archaeology. Dendrochronology, although not yet a commonplace technique, already is shedding light on such wide ranging subjects as past climates, sea level changes, tree ecology and forest management in prehistoric and historic times, and is proving an invaluable aid in dating of archaeological remains and in art history.

Holding the meeting in London was particularly important for until the past 10 years or so little research on tree-ring analysis has been done in Britain, although some pioneering work was done in the 1940s and 50s by Lowther and Schove. This slow start may be explained partly because of the difficulty of devising suitable reference chronologies for oaks growing in southern and eastern England, but also partly by unfamiliarity with the classical German and Russian work on the subject.

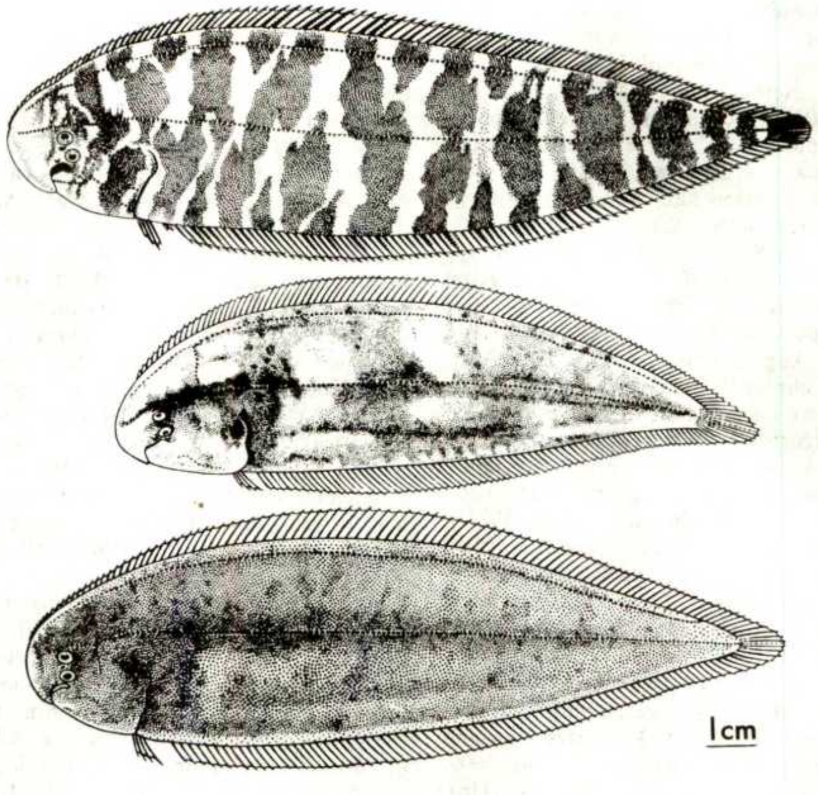
The problem in the development of tree-ring analysis in north-west Europe has been that the growth of deciduous trees and thus the pattern of annual rings is more variable in temperate regions than in the semi-arid and arid areas of south-west USA: site conditions (such as altitude, and soil), defoliation by insects and man's influence may affect tree growth in Europe as much as temperature and rainfall—the predominant

Tongue soles revised

from our Marine Vertebrate Correspondent

THE flatfishes are an interesting and important group of bony fishes: interesting because of their unique asymmetry which involves a complicated reordering of the head skeleton, eyes and optic nerves on metamorphosis; important because almost throughout the world they are of considerable value as food fishes. These two aspects have resulted in their being studied by workers in different disciplines possibly more than most comparably sized fish groups. Their systematic arrangement and taxonomy have been studied, notably by Norman in his *Systematic Monograph of Flatfishes* **1** (London, 1934) although he never completed the second volume which was to have included the families of true soles and tongue soles (Soleidae and Cynoglossidae).

To some extent this hiatus has partly been filled by a comprehensive revision of the tongue soles of the genus *Cynoglossus* by A. J. K. Menon (*Smithsonian Contributions to Zoology,* no. 238, 1–129; 1977). The Cynoglossidae are flatfishes which have a typical elongate body form, tongue sole being a most apt name. The dorsal, caudal, and anal fins are continuous around the edge of the body, other fins are reduced or absent, and the mouth is placed on the side of the head, in most species having a sinister curve about it. In the genus *Cynoglossus* the eyes are very small, close together and are always on the left side of the head. It seems that the reduction of the eyes is probably associated with the well-developed lateral line sensory system; in some species three lateral lines run along the length of the body while most have a complex of head lines. On the blind side, however; this sensory system is reduced or totally absent (a striking difference to the situation in the true soles which have a highly evolved sensory system on the eyeless side of the head). As Menon points out members of the Soleidae are mostly feeders on the surface of the sea bed, while the tongue soles are adapted to plough through the mud or sand on the bottom, hidden beneath the surface themselves, and feeding on buried food organisms. As an adaptation to this way of life the cynoglossids do not have the fin rays of the dorsal fin running forward to the extreme edge of the head (as do the soles) but the first dorsal pterygiophore, which in other fish would support the anterior fin rays, is modified to form a rostral cartilage. This rostral cartilage provides support for the thin, elongate snout which acts like a forward-pointing ploughshare as the fish slices its way through the seabed.



Three specimens of *C. puncticeps* showing variation in pigment pattern. Top from Kerala; others from Cochin.

Menon's revisionary study is, of course, basically a systematic work. He gives a brief discussion of the other genera in the family (*Symphurus* with forty or so species, *Paraplagusia* with only four species), and then detailed diagnoses of the forty-nine members of the genus *Cynoglossus.* All the species are illustrated by simple line drawings supplemented by photographs of most species. Menon's work thus means that in the future there will be an adequate source of identification available for these closely similar fishes.

The geographical distribution of these tongue soles has a number of points of interest. The centre of distribution lies in the eastern Indian Ocean–West Pacific area, especially around the Malay archipelago, where Menon finds the more specialised and rapidly evolving species complexes. Around the periphery of this region more primitive types are found, ranging from Japan to eastern Australia, and along the Indian Ocean coast of Africa. *Cynoglossus* is one of the few groups of fishes which are widespread in the Indo-Pacific and are found off the West African coast as well, although absent in the Western Atlantic. Most of the West African species belong to a large group of species (the *canariensis* group) which Menon regards as primitive and near to the ancestral stock. Menon's study thus has some interest in the evolution of this group of flatfishes and will be of value to zoogeographic studies although work in a broader field than at generic level will be necessary to evaluate some of his conclusions concerning relationships and distribution.

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factors in arid and tropical countries —and so make it difficult in parts of northern Europe to devise reference curves for trees such as oak, beech and elm which all react differently to environmental conditions. In northern Europe too there is the added disadvantage that no tree has the longevity of the bristle-cone pine or sequoia although sessile oaks often live more than 500 years. Oak is the timber found most often in archaeological excavations.

Although the Russian, Shvedov, as long ago as 1892, related variations in annual ring width to rainfall, it was the German, Huber, who from the 1940s to his death in 1970, laid the foundations of the modern methodology and his influence remains in the rigorous work of J. Bauch and D. Ekstein (University of Hamburg) and B. Becker (University of Hohenheim) which sets standards for all other laboratories. Computer programs devised by Bauch and Ekstein now make it possible to cross-date local and regional tree-ring curves reliably. A master chart established for oak in the Netherlands dating back to AD 1036 synchronises with corresponding curves in southern and western Germany where, as Becker reported, there is now a master chronology from the present back to 717 BC.

In northern Germany and in the coastal region of the North Sea from Denmark past the Netherlands and Flanders to south-east England and East Anglia, however, the major German chronology does not usually apply and so independent curves have been constructed. For Haitabu in the Schleswig area of north Germany, where dendrochronology has been used to date the important Viking trading centre, Ekstein has established a chronology dating back to AD 400. In the Netherlands Bauch has found a second chronology, independent of his other, which covers the years AD 1109–1637. To this curve are related parts of eight overlapping chronologies, each about 300 years long and covering the period AD 514 to 1609, established by J. M. Fletcher (University of Oxford) for southern and eastern England.

Both Fletcher and Bauch have used a combination of excavated and architectural timbers and oak panels of paintings to make their reference curves. An added dimension to this work is its relevance to art history. In England, for example, oak panels were the common means of support for most portraits painted before 1620. By classifying 150 such paintings dendrochronologically, Fletcher has been able to gather information about panels from the same tree and to deduce the likely date to within 10–20 years of some of the portraits whose history is uncertain. Bauch, similarly, has studied over 200 Dutch and German paintings mainly of the seventeenth century.

Other chronologies for Britain are gradually being established. P. Leggett and A. Hibbert (Liverpool Polytechnic) are working on trees felled in woodland in North Wales and have a master chronology for oak running from AD 1711 to 1975 which cross-dates with one in Northern Ireland prepared by M. G. L. Baillie (Queen's University, Belfast). R. Morgan (University of Sheffield) is dating material excavated by J. Schofield (London Museum) from Roman and Mediaeval sites on the River Thames in London. Tree-ring analysis of this timber, recovered from old waterfronts, is helping to clarify discrepancies between the dates inferred from the stratigraphical position of artefacts found on the site and radiocarbon dating. Valuable topographical clues are also being obtained from the way the timber was used in the construction of the waterfronts, and changes in the environmental conditions around London from Roman to Mediaeval times can be suggested by study of the ring-widths of the oak timbers. When two species, oak and elm, are found together in Mediaeval excavations, then comparisons of their use and growth at a particular time have been made (D. W. Brett, Bedford College, London). In another part of England an oak chronology for the period AD 1635–1972 has been established for the Winchester area (A. C. Barefoot, North Carolina State University, and others) and this curve, when combined with ^{14}C dates and a separate chronology for earlier timber, is being used to date excavations in the city led by M. Biddle (Winchester Research Unit).

Across Europe in East Germany, M. Jährig (Akademie der Wissenschaften der DDR, Berlin) has examined pieces of timber excavated from Slavonic settlements dating from the 6th to the 10th centuries AD with the aim of establishing a master chronology for GDR. So far he has obtained a relative chronology for 492 years in this period in the central region of the country; a separate chronology will probably be needed for the northern region.

In Sweden, there is now a master chronology for oak for the past 1,400 years. Analysis of oak used to make coffins excavated from Mediaeval churchyards in Lund (T. S. Bartholin, University of Lund) is suggesting how the climax forest in that part of Scandinavia changed during the period AD 1000–1200. In the earlier coffins the oak boards are narrow ringed and range up to 500 years old; but by AD 1150 the oaks had few and broad rings, suggesting that by this date open woodland pasture had replaced the dense virgin forest.

Much earlier chronologies using timber and radiocarbon dates are being devised for the Somerset Levels, a wetlands area in south-western England where an extraordinary series of wooden trackways of various constructions have been excavated by an inter-university team led by J. M. Coles (University of Cambridge). A member of this team, B. J. Orme (University of Exeter), described how tree-ring analysis is being used to establish dates for the substantial oak planks and for the brushwood material of ash, hazel, alder and birch used in the building of the trackways which date from the fourth to the first millenium BC. Analysis of this worked wood at different sites has enabled the team to determine which woods were used for which purpose in the trackway structures, to show that woodland management by coppicing existed as long ago as the fourth millenium BC and to ascertain the durability and re-use of different timbers. Past environmental conditions can also be inferred from studies of the timber from different millenia.

Other applications of dendrochronology reported included a study of submerged forests around the British Isles by A. Heyworth (University College of North Wales, Aberystwyth) who is using tree stumps ranging in age from more than 8,000 years to less than 1,000 years as a measure of sea level changes. R. E. Tout and W. E. Gilroy (University of Surrey) are analysing concentrations of trace elements in tree rings: the technique offers a way of distinguishing the heartwood and sapwood boundary where this is unclear. The elements potassium, chlorine, and manganese seem useful as indicators.

Most dendrochronologists use measurements of ring width to prepare their reference curves. Another line of approach is to measure wood density by X-ray densitometry and two speakers, F. H. Schweingruber (Swiss Forest Research Institute, Birmensdorf) and H. Polge (Centre National de Recherches Forestières, Champnoux, France) believe that the technique is a better indicator than ring width of climatic changes in the past and is quicker to use. Schweingruber is using subalpine conifers (fir, spruce, pine and larch) to reconstruct the fluctuations in summer temperature in the Alps. S. J. Milsom and M. K. Hughes (Liverpool Polytechnic) are experimenting with X-ray densitometry on oak which seems to present more problems than pine although it is thought that the technique will be useful for dating wide ringed timber.

On the third day of the meeting, the point made by G. C. Varley (University of Oxford) that defoliation of oaks by caterpillars can have similar effects on tree growth as harsh weather led to lively discussion and reminded everybody that the wood they work with came from a living tree whose growth was influenced by a variety of quantifiable and unquantifiable factors, not forgetting drought, plagues of insects, sunspots and other 'Acts of God'. These may be 'noise' but should not be disregarded in constructing computer programs. Computers and statistics, although essential tools today, must be used discriminately; as several participants pointed out the human eye and brain remain indispensable for noting anatomical irregularities in timber and for the matching of tree-ring curves. □

review article

Radio sources with superluminal velocities

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Radio data from four extragalactic sources, three quasars and one galaxy, show evidence for an apparent expansion faster than the speed of light. The data on these 'superluminal' sources are reviewed, and their implications briefly discussed.

BRIGHTNESS distributions in four extragalactic radio sources have been seen to vary so rapidly that the apparent transverse velocity of expansion is greater than the velocity of light (assuming a cosmological origin for the redshift). The term superluminal will be used to describe this phenomenon. In this paper we review many of the observations of superluminal expansions, and also add some new material. Blandford, McKee, and Rees[1] have summarised theoretical ideas on the subject.

The observations have all been obtained with very-long-baseline interferometry (VLBI) systems using two to five radio telescopes, spaced over thousands of kilometres, to form multi-element interferometers. Various model-fitting and map-making procedures have been used to estimate parameters of the brightness distribution, such as diameter, separation and flux of components, position angle, and so on. In some cases the data have been sufficiently accurate and extensive to warrant many-parameter models, and detailed contour diagrams have been made. In general, however, the models are not unique. Some have been challenged[2,3]; but there still is general agreement among different workers on the main features of the sources. VLBI map-making is presently in a stage of rapid development; in particular there is a growing use of phase data, and more definitive results are expected in a few years.

Four sources including three quasars (3C345, 3C273, and 3C279) and one radio galaxy (3C120) will be discussed. In each case we give simple angular measures and do not discuss the detailed models of brightness distribution. More complete discussions will be found in the references.

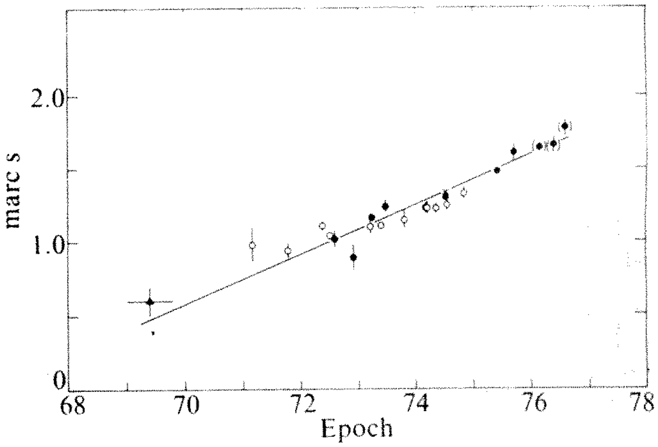
3C345 ($z = 0.595$)

Two observing groups[4,5] have independently shown that 3C345 could be accurately described as a simple double source in mid-1974. The two components were approximately Gaussian and contained most or all of the flux density at $\lambda = 2$, 2.8, and 3.8 cm. In these experiments three or four telescopes were used. The simple model was an excellent fit, and there was no requirement for, nor evidence of, a more complex structure. At most other epochs only two or three telescopes were used. The brightness distributions were not as well determined, of course, but a simple double fit the data and the separation, θ, was accurately determined in every case.

Figure 1 shows the separation of the centres of the two components as a function of time. The point at 6 cm is taken from a number of measurements made in 1968 and 1969 (ref. 8). The source was only slightly resolved at that early epoch and θ was determined from the best-fitting double. The position angle was assumed to be 105°, the value found more recently. The 3.8 cm point at 1971.1 (ref. 7) is similarly model dependent because the source is only partially resolved. The three points in 1976 are provisional because detailed model fitting is yet to be done; however, they are unlikely to change much because θ is well determined from the sharp minima of the visibility function.

The points in Fig. 1 show that the brightness distribution expanded by a factor of about 3 in 7 years, at the approximate

Fig. 1 Apparent separation of the two components of 3C345 as a function of time. The slope of the line is 0.17 marc s yr^{-1}. The observations at 2 cm are taken from ref. 5; the 2.8-cm points are from refs 5 and 6, and the four points in 1972–73 are from private communications with N. Broten and M. Quigley (the points in 1976 are discussed in the text); the 3.8 cm points are from refs 4 and 7; and the 6-cm point is discussed in the text. ×, 2.0 cm; ●, 2.8 cm; ○, 3.8 cm; ▲, 6.0 cm.



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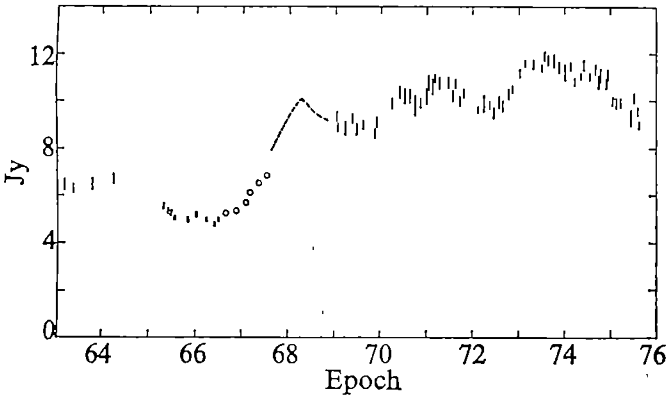


Fig. 2 Total flux density of 3C345 at $\lambda = 3.8$ cm. Points from 1963 to 1966.5 are taken from ref. 10; from 1966.6 to 1967.5, ref. 11; 1969.0 to 1971.6, ref 12; 1971.6 to 1974.4, ref 13; 1974.4 to 1975.6 from private communications with W. A. Dent, and the dashed line is interpolated between observations at 2.8 and 4.5 cm reported in ref. 14.

rate 0.17 marc s per year. The expansion may not be uniform, however, for the slope seems to be flatter in 1972–73, and steeper in 1975–76. The difference in slope explains most of the discrepancy in the rates already reported in the literature[8, 9]. The angular rate can be converted into an apparent transverse velocity at the source by multiplying by the cosmological distance and correcting for time dilation with a factor (1+z).

$$\frac{v}{c} = \frac{\theta}{H_0 q_0^2 (1+z)}\left(q_0 z + (q_0 - 1)[(1+2q_0 z)^{\frac{1}{2}} - 1]\right)$$

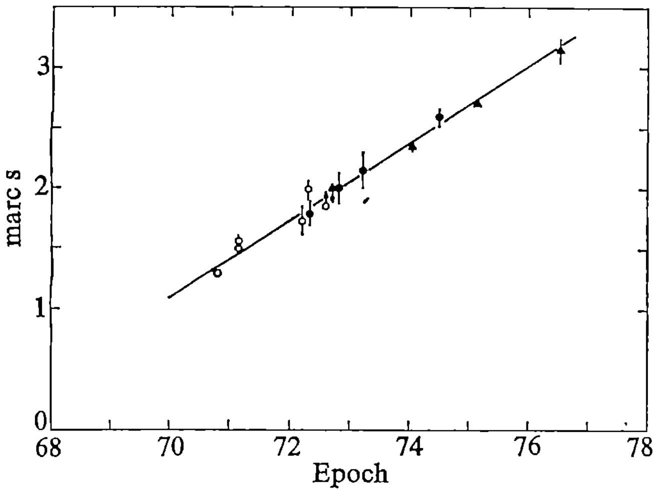
The line in Fig. 1 corresponds to $v/c \approx 7$. ($H_0 = 55$ km s^{-1} Mpc^{-1} and $q_0 = 0.05$.)

The angular separation of the components of the double seems to be independent of wavelength over at least an octave. However, the detailed visibility curves show that the ratio of flux densities varies with wavelength; that is, the components have different spectra.

The position angle (PA) of the double was determined for all cases shown in Fig. 1 for which there were adequate data. There are no substantial differences with wavelength or time; the PA has been constant at 105°±3°.

Extrapolation of the rate shown in Fig. 1 to zero separation indicates that an 'event' occurred in 1966. Figure 2 shows the total flux density of 3C345 at 3.8 cm. A major increase in flux density began in 1966; the flux doubled in two years and has since stayed high. The near coincidence of the times suggests that the outburst and the expansion have a common origin.

Fig. 3 'Size' of 3C273, where size $\theta^* = (2w)^{-1}$ and w is the distance to the first minimum of the visibility function. The slope of the line is 0.32 marc s yr^{-1}. The observations at 2.8 cm are taken from refs. 15 and 16 and the points at 3.8 cm are taken from refs 7 and 17–19. The 6-cm points are our previously unpublished data. ●, 2.8 cm; ○, 3.8 cm; ▲, 6.0 cm.



3C273 ($z = 0.158$)

3C273 has a complex elongated structure (refs 3, 15 and 16 and W. D. Cotton *et al.*, in preparation). Its low declination (2°) means that the (u,v) coverage is poor and the models are more indeterminate than for high declination sources like 3C345. In consequence, different authors have proposed models of different character, some expanding with time[7,15], and others with stationary components whose intensities are suitably varied[3,17]. All observers agree that the overall size of the brightness distribution increased from 1971 to 1974, but the uniformity and nature of the increase has been argued.

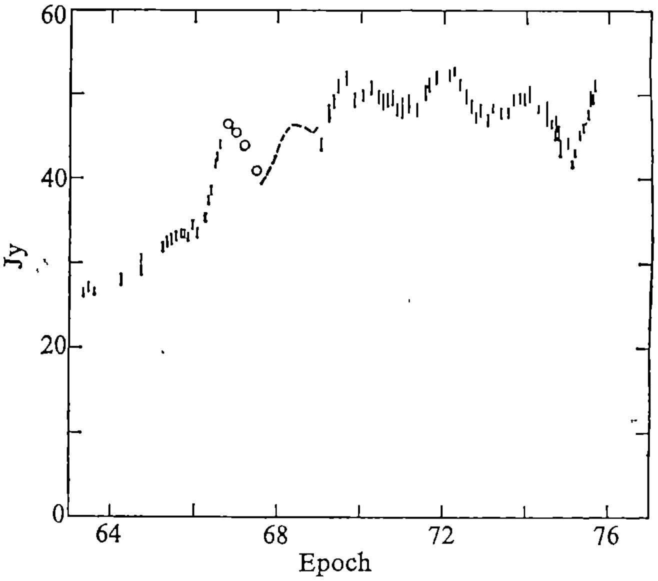


Fig. 4 Total flux density of 3C273 at $\lambda = 3.8$ cm. References for the points are as in Fig. 2.

Figure 6 of Schilizzi *et al.* (ref. 15) shows a succession of (u,v) diagrams with the observed lines of maxima and minima of the visibility function. The lines are not equally spaced; therefore, the source cannot be represented by a simple (that is, Gaussian) double. Furthermore, the relative spacings change with time, and successive sets of lines move toward the origin. This means that the shape changes with time, and that, in some measure, it is increasing in size. The visibility data shown by Schilizzi *et al.*, however, do not define the brightness distribution uniquely.

In an attempt to escape difficulties caused by modelling with inadequate data, we have studied one simple measure of the overall size, the distance from the origin to the first minimum of the visibility function. Let w (in wavelengths) be this distance, then $\theta^* \equiv 1/(2w)$ is the angular separation of the components if the source is a simple double. In the more general case where the source consists of several isolated components strung out on a line, θ^* is a good measure of the overall angular scale. For example, in a wide range of triples which we studied numerically, θ^* varies from 1.0 to 0.65 times the overall size.

We plot θ^* at various epochs in Fig. 3. Many of these observations were taken on only one baseline, and the position angles were very poorly determined. So, to provide a consistent basis for calculating θ^*, we assumed PA = 64° in all cases. This is the typical value obtained when multi-baseline data are available, although the source is known to be nonlinear[3,16]. The error bars in Fig. 3 come from the uncertainty in estimating the locations of the minimum, and do not include any errors from an incorrectly assumed position angle. The arrows in mid-1972 signify lower and upper limits.

Further data exist at 2.8 cm for 1975 and 1976, but the character of the visibility function is different then from what it was earlier. The functions do not contain any clearly recognisable extrema (except at the origin), but drop rather smoothly to a 'core' value. The brightness distributions may still contain 'components', but all (or perhaps all but one) of them must be large enough to be individually resolved at the interferometer spacings which would otherwise produce maxima and minima. In contrast, at 6 cm the structure still had concentrations which could produce the observed minima. These recent results will be discussed separately.

The 2.8-, 3.8-, and 6.0-cm data all fit together very well up to 1975. We conclude that the basic shape was independent of wavelength over this range, although there were spectral differences in component intensities.

The overall size of the brightness distribution increased rather steadily and more than doubled in 6 years. The word 'expansion' may properly be used to describe this behaviour. The points in Fig. 3 cluster around a line with slope $\theta^* = 0.32$ marc s per year ($v/c = 4.2$). If the line is extrapolated it hits zero in 1966. The total flux density at $\lambda = 3.8$ cm is shown in Fig. 4, and, again, the 'expansion' starts, roughly, near the beginning of a large sustained increase in flux density.

3C120 ($z = 0.033$)

3C120 varies rapidly in flux density and appearance, and has been observed frequently in an attempt to follow its variations[7,15,21-25]. Although many of the observations have been rather incomplete, in several cases very good data were obtained with 3 or 4 telescopes, and simple double models fit very well. The best of these cases comes from our observations at 2.8 cm in 1976.1, when 5 well defined maxima and minima were observed using telescopes at Green Bank, Fort Davis and Owens Valley. As with 3C345, a well-separated Gaussian double accurately fit all the visibility data. However, the compact structure in 3C120 never produced nearly all the total flux, the way it did in 3C345. The compact components of 3C120 are embedded in a larger, undetermined structure.

In all cases with good data the PA was near 65°. To determine a size in the poorer cases, we assumed that a double was always a reasonable representation of the source; and, when necessary, we also assumed PA = 65°

The separation of the two components of the double is plotted in Fig. 5 for all values in the literature (with exceptions noted below) together with some of our previously unpublished data at 2.8 and 6.0 cm. The parentheses indicate cases where there was ambiguity; typically, the source was only weakly resolved, or the size determination was made from a single minimum on one baseline, or the first minimum was not directly observed.

We recognise two major epochs of expansion in Fig. 5, and have indicated them with straight lines whose slopes correspond to $v/c = 5$ and $v/c = 8$. At 1974.5 the 3.8 and 2.8 cm observations showed doubles of very different separation. No unique interpretation can be given for this, for the size and strength of the components of the doubles are very poorly determined. The observed double structure did not account for the total flux at either wavelength, so it is possible that both doubles existed at both wavelengths but were not recognised because of the differing resolutions. Thus, the close-spaced double seen at 2.8 cm might not have been recognised at 3.8 cm because its first minimum would have come at 1.9×10^8 wavelengths, whereas the maximum length of the baseline was only 1.0×10^8 wavelengths. Similarly, at 2.8 cm where the minimum length of the baseline was 1.6×10^8 wavelengths, components larger than about 1.5 marc s would have been resolved, and this double would have been missed. Spectral differences of the type apparently seen in 3C273 would increase the size of the outer components at 2.8 cm and accentuate this behaviour.

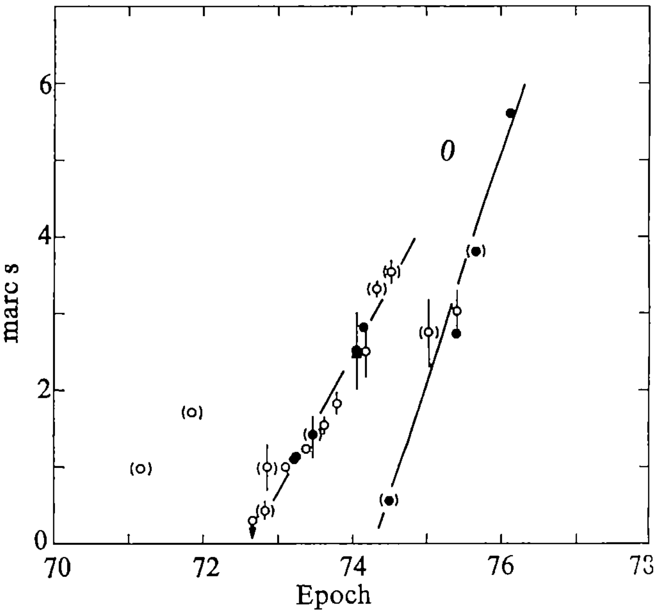


Fig. 5 Separation of the two components of 3C120, assuming a double at PA = 65° when necessary. The slopes of the lines are 1.8 and 2.9 marc s yr^{-1}. The early observations at 2.8 cm are from ref. 15, and the points from 1974.5 on are previously unpublished. The 3.8-cm data are from refs 7, 15, 21-25. The 6-cm point at 1974.5 is previously unpublished. ●, 2.8 cm; ○, 3.8 cm; ▲, 6.0 cm.

The points in 1971 were once interpreted as evidence for a superluminal expansion[21], but that result is ambiguous with only two points. Further data exist for early 1972 (refs 22, 23), but they are not plotted. The visibility was changing rapidly then, but remained very low. Therefore, the strong concentrations which must have existed in 1971 had dissipated, and by 1972.3 most of the flux came from regions greater than about 1 marc s in diameter.

During both epochs of expansion the PA was near 65° whenever a good model could be generated.

Fig. 6 Total flux density of 3C120 at $\lambda = 3.8$ cm. Points from 1969 to 1971.6 are taken from ref. 12; 1971.6 to 1974.4, ref. 13; 1974.4 to 1975.6, from private communications with W. A. Dent, and the dashed line is interpolated between observations at 2.8 and 4.5 cm reported in ref. 14.

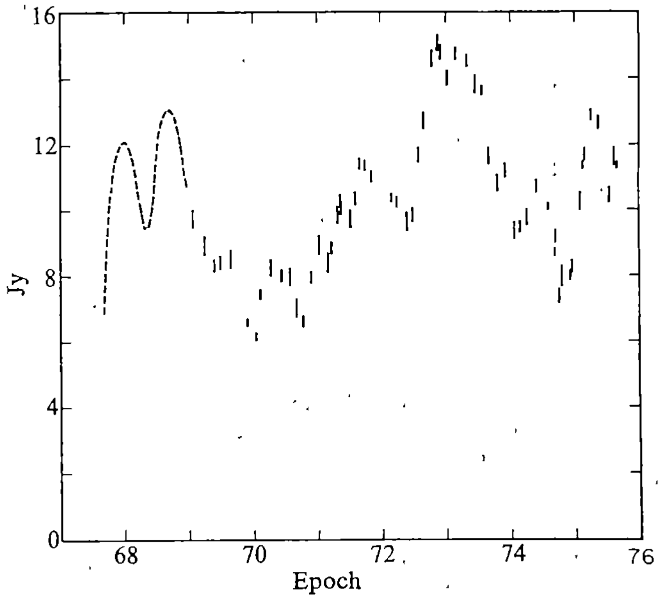


Table 1 Strong compact extragalactic radio sources

Source	Name	Id	z	$S_{3.8}$ (Jy)	VLBI	Refs
0316+41	3C84	G	0.018	20-58	slow variable	15, 24, 25, 27, 28
0355+50	NRAO150			7.3-15	stable double (?)	16, 24, 29
0430+05	3C120	G	0.032	6.4-15.1	superluminal	
0923+39	4C39.25	Q	0.699	8.5-11.8	stable double	5, 24, 29
1226+02	3C273	Q	0.158	28-53	superluminal	
1253−05	3C279	Q	0.538	11.2-17.5	superluminal	
1641+39	3C345	Q	0.595	8.8-11.9	superluminal	
2134+00		Q	1.94	11.0-13.4	slow variable	15, 24
2200+42	BLLac	G	0.07	4.8-14.2	rapid variable	16, 24, 29, 30
2251+15	3C454.3	Q	0.859	9.0-20.2	variable core	16, 24, 26, 29

Figure 6 shows the total flux density of 3C120 at $\lambda = 3.8$ cm. This source varies more rapidly and has a greater fractional variation than 3C345 and 3C273. The largest outburst in Fig. 6 begins in mid-1972 and is coincident with the start of the first expansion shown in Fig. 5. The second expansion starts in early 1974 and does not appear to be correlated with any exceptionally large flux event.

3C279 ($z = 0.538$)

The available data on 3C279 are mainly at $\lambda = 3.8$ cm, from the period 1970–73 (refs 7, 17–20, 26). Most of this material is from only one baseline, and the interpretations are ambiguous.

The two earliest observations have been interpreted as an expanding double, with separation rate $\dot{\theta} = 0.27$ marc s yr^{-1} ($v/c = 10$) (refs 7, 19). The rate is not well determined with only 2 points, but it is consistent with observations at 6 cm in early 1972 (ref. 17), which give the overall size as about 2 marc s. A minimum in the 13-cm visibility function on baselines from California to Australia[26] near the beginning of 1970 is also consistent with a simple double model based on the 3.8-cm observations, suggesting that the separation of the two components was similar over that range of wavelengths.

A sequence of observations at 3.8 cm in 1972 (ref. 17) can be interpreted as a new expansion event, since an unresolved weak core was seen in March and April 1972, and this became substantially resolved by November 1972. Another possibility is that all the 1972 data came from a triple source whose overall dimensions were constant, but whose component flux densities varied[17]. The published material is consistent with either model, and further data are necessary to discriminate between them.

Interpretatations

It is of interest to ask what fraction of all sources are superluminal, and whether statistical arguments can be made for or against any of the customary interpretations. These are difficult questions because adequate surveys do not exist at the short centimeter wavelengths, and VLBI surveying is particularly incomplete. However, we can make some comments.

At $\lambda = 3.8$ cm, 3C345 is the weakest known superluminal source, with mean flux density $<S_{3.8} \sim 10$ Jy during the expansion. We know of 15 extragalactic sources which have been as strong as 10 Jy at 3.8 cm, at any epoch. Five of these are radio galaxies with most of the flux coming from extended regions. (3C123, 3C353, Vir A, Cen A, Cyg A). The remaining 10 sources are shown in Table 1. The fifth column in Table 1 gives the known range of flux density at $\lambda = 3.8$ cm. The sixth column gives a comment on the structure seen with VLBI, and the seventh column gives references for those sources not discussed in detail above.

3C84 has several components which vary in flux density but seem to have little motion. NRAO150 has been observed less than the others and its size is less than 0″.001 so that intercontinental observations are necessary for reasonable resolution.

4C39.25 has a well-defined double structure with constant separation, but the total flux density is variable. 2134+004 apparently is more complex than a simple double, and has slow weak variations in its brightness distribution. BL Lacertae has a double structure which is variable but keeps a constant position angle. The observed changes in BL Lac are not systematic as in the superluminal sources, but it may well be that shorter sampling intervals are required to see them, as this is the most variable source in Table 1. 3C454.3 has a core-halo structure. The variations in total flux density follow those of the unresolved core.

Nearly half the strong compact sources show a superluminal effect. This suggests that mechanisms which require us to be in an especially favourable position are unlikely to be at work. Luminous relativistically moving clouds are one such class, because to see them superluminally we have to be within a small solid angle[1]. However, the blue-shift of the approaching clouds also raises the possibility of a selection effect. Perhaps superluminal sources seem common partly because the blue shift raises their flux density above the minimum level being counted. To study this possibility we need to know the distribution of superluminal sources with flux density, which is unknown at present.

The superluminal sources all show a systematic expansion, and at least one of them shows two epochs of expansion with the same PA. This argues strongly against mechanisms which basically are random and allow both expansions and contractions. Gravitational lenses and some other propagation and opacity effects are of this type. So, too, would be a collection of separated sources which are independent, and flash at random times. Propagation and opacity effects also are unlikely because they predict that the separation would be wavelength dependent, contrary to observation.

The fact that the superluminal effect has been seen in a galaxy as well as in three quasars suggests that it cannot be used as evidence against a cosmological origin for the redshifts.

Thus what is now known can be summarised as follows. Nearly half the strong ($S_{3.8} \geq 10$ Jy) compact ($\theta < 0''.01$) sources show a superluminal expansion at centimetre wavelengths, and changes in overall size of up to factor 10 in periods of one to two years have been observed.

No systematic contractions have been seen. The separation of components in a source is basically independent of wavelength from 2 to 6 cm, although the components may have different spectra. In 3C273, and perhaps in 3C120, we may have observed an epoch where component evolution produced different sizes at different wavelengths.

The position angle is substantially constant during an expansion. In at least one case (3C120) there have been two distinct epochs of expansion. The position angle is the same for both.

The brightness distribution does not expand uniformly, but there is an overall trend as shown in the graphs. The epoch of zero separation, determined by extrapolation of the trend, may be close to the start of a major outburst in flux density.

The extensive data for 3C345 at 1974.5 and for 3C120 at 1976.2 strongly suggest a structure which can be represented as a simple double, with the two components being approximately equal. 3C273 is more complex. There are both simple (4C39.25) and complex (3C84) sources which do not show variations in the overall size of the brightness distribution although their total flux densities vary on time scales similar to those for the superluminal sources.

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articles

Percolation theory of residual phases in porous media

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When one fluid displaces another by flow through a porous medium, the fraction of pore space occupied by trapped residual phase correlates with the ratio of viscous to capillary forces in the flow. This dependence, heretofore unexplained, is derived from the mechanics of fluid blobs, the nature of pore structure, and percolation theory. Pore space topology is shown to be as important as geometry in the statistical flow behaviour of large populations of fluid blobs in two-phase flow in porous media. Topology and geometry can each be modelled usefully by a single parameter characteristic of the pore space.

WHEN porous solids, whether unconsolidated such as powders, granulated solids, soils, and clastic sediments, or cemented and often diagenetically altered, such as bones, sintered solids and sedimentary rocks, have their pore space occupied by two immiscible fluid phases at least two connectivity domains of the non-wetting phase can be observed. In one—'the continuous regime'—the phase is largely self-connected over the range of many pore diameters, and in the other—the 'blob regime'—the phase exists only as discrete, disconnected 'blobs' surrounded completely by the other phase[1]. The wetting phase, of course, can also exhibit continuous and discontinuous regimes, but these will not be considered here.

The saturation of a phase is defined as the fraction of pore space or void space occupied by that phase. It is observed in flow experiments that when a non-wetting phase in the continuous regime is drained to a saturation below some critical value (about 0.1–0.5 (ref. 2), depending on the fluids) by the flow of a second, wetting, phase, the former phase breaks up into discrete blobs. In this blob regime, the non-wetting phase is much more difficult to displace from the porous medium. There has previously been no satisfactory theory of these facts, nor of related phenomena of capillary pressure and relative permeability, although some of the elements for the explanation have been available. These constitute blob mechanics and are reviewed and extended here. They are then tied together with new elements from percolation theory. The synthesis makes it plain that the topology and randomness of the pore space are important. In this article, we show how these aspects influence residual saturation.

In typical porous media, a fluid at a given saturation in the continuous regime flows with flow rate proportional to the applied pressure gradient (Darcy's Law[1]) even for very low pressure gradients. A fluid phase in the blob regime, however, does not flow at all at a given pressure gradient, if γ, the interfacial tension between the two phases, exceeds a certain critical value.

This contrast is easily explained by reference to the Young–Laplace equation for the pressure jump across an interface between static fluids

$$\Delta p = \gamma\left(\frac{1}{R} + \frac{1}{R'}\right) \qquad (1)$$

where R and R', the principal radii of curvature of the interface, are, for fluids in a solid matrix, complicated functions of pore geometry and the contact angle which the interface makes with

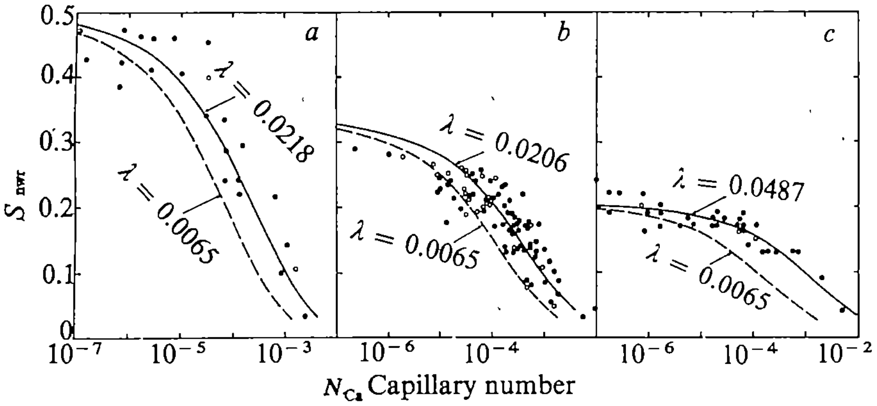


Fig. 1 Residual non-wetting phase saturation as a function of capillary number, experiment and theory. *a*, Foster and McMillen's data, $\sigma = 2$; *b*, Abram's data, $\sigma = 3$; *c*, Lefebvre du Prey's data, $\sigma = 5$.

the pore wall. Nevertheless, R and R' are of the order of D, a characteristic pore diameter of the medium; thus

$$\Delta p \sim \frac{\gamma}{D} \tag{2}$$

For typical values of γ (30 dyne cm^{-1}) and D (10^{-4} cm) Δp is of the order of 3×10^5 dyn cm^{-2}, or about a third of an atmosphere. A blob in static equilibrium in a typical porous medium at ordinary values of γ can, therefore, develop large pressure differences across its interfaces. If equation (1) holds at least approximately during slow flow, then with such large pressure differences, disconnected blobs fail to flow because they easily adjust their meniscus configurations to withstand low imposed pressure gradients. According to blob mechanics, the adjustments required are greater the longer the blob is in the flow direction. Hence, longer blobs are more readily mobilised than shorter ones. These predictions have recently been confirmed experimentally for the first time[3].

Residual saturation should thus also depend on the distribution of blob lengths, which is a consequence of the way in which the continuous regime breaks down into the blob regime. Because this process seems to be governed by randomness of the pore space, we adopt the hypothesis that the size distribution of blobs is that of one material randomly mixed with another within a space which is conveniently approximated as network-like or tree-like. Applied to this hypothesis percolation theory yields the needed size distribution. The resulting predictions of residual saturation agree with extensive experimental data hitherto unexplained.

Blob mechanics

Imagine a blob trapped in a porous sample surrounded by continuous fluid which is under an imposed average pressure gradient, dp/dx. In what conditions will this blob be mobilised? To answer this precisely, requires finding the conditions in which the given fixed-volume blob can find no configuration which satisfies the Young–Laplace equation and the boundary conditions imposed by the geometry of the pore space it is in. To identify these conditions approximately, we observe that the viscous flow of continuous fluid around the motionless blob develops a pressure difference, $\Delta p_{\text{avail}} \simeq l dp/dx$, across its length, l, in the flow direction, which is available to drive the blob forward. The pressure difference necessary to dislodge the trapped blob, Δp_{nec}, is given approximately by $\Delta p_{\text{nec}} = \alpha\gamma/D$, where α is a dimensionless constant which depends on the geometry of the trapping pore space. The blob is mobilised when

$$\Delta p_{\text{avail}} \geq \Delta p_{\text{nec}} \tag{3}$$

and the minimum blob length, l^*, required for mobilisation, is given by the condition $\Delta p_{\text{avail}} = \Delta p_{\text{nec}}$, which can be rewritten as

$$l^* \simeq \alpha\gamma/D \left(\frac{dp}{dx}\right) \tag{4}$$

Now dp/dx is proportional to the continuous phase fluid velocity, v_w, according to the extension of Darcy's Law which accounts successfully for two-phase flow[1]

$$v_w = \frac{k_{rw}K}{\mu_w}\left(\frac{dp}{dx}\right) \tag{5}$$

μ_w is the continuous phase fluid viscosity; k_{rw} its relative permeability at the specified saturation, and K the permeability of the porous medium, so that

$$l^* = \left(\frac{\gamma}{v_w}\mu_w\right)\frac{\alpha k_{rw}K}{D} \tag{6}$$

A reasonable approximation which has proved useful for many porous media is[4]

$$D \simeq \beta\sqrt{K\varphi} \tag{7}$$

where φ is the porosity of the medium and β is a dimensionless constant. Letting $n^* \equiv l^*/D$, a dimensionless blob length, gives

$$n^* \simeq \lambda\left(\frac{\gamma}{v_w\mu_w}\right)k_{rw}\varphi \tag{8}$$

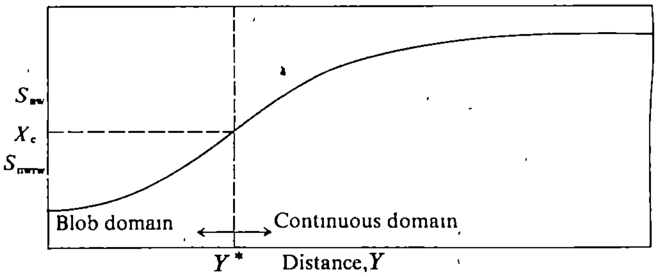
where $\lambda \equiv \alpha/\beta^2$. If only continuous phase is flowing, $v_w = v$, the superficial velocity, and

$$n^* \simeq \lambda N^{-1}{}_{Ca}\, k_{rw}\varphi \tag{9}$$

where $N_{Ca} \equiv v\mu_w/\gamma$ is a dimensionless ratio of viscous mobilising forces to capillary trapping forces.

An analysis of this sort was also made by Melrose and Brandner[2], who derived our λ in terms of pore geometry and

Fig. 2 Schematic saturation profile in a porous medium at a point in time during displacement of a non-wetting phase.



blob configuration. Using estimates which they reported from experiments on unconsolidated packs of uniform, nearly-spherical glass beads or sand grains, we find an approximate value for λ is 0.0065. This microscopic theory of blob mobilisation, following Melrose and Brandner, predicts that for a given blob there is a critical capillary number above which it cannot remain in static equilibrium, a prediction confirmed by experiments with individual blobs[3].

Blob population dynamics

The theory of the relationship between the mechanics of individual blobs and the flow behaviour of large populations of them has been missing. Their collective, macroscopic flow behaviour has been investigated experimentally, however. The results show a correlation between the capillary number, N_{Ca}, appearing in equation (9) and S_{nwr}, the residual non-wetting phase saturation which is irremovable at the given flow conditions no matter how much driving fluid is flushed through the solid matrix. Several investigators[2,5-9] reporting on a large number of experiments using a wide variety of fluids in porous media, have shown that at values of N_{Ca} less than about 10^{-7}, S_{nwr} is a constant in the range 0.2–0.5. As N is$_{Ca}$ increased past a value in the range 10^{-7} to 10^{-5} (different investigators report different values) S_{nwr} begins to decrease. As N_{Ca} is increased further, S_{nwr} continues to decrease and eventually reaches zero at a value of N_{Ca} between 10^{-3} and 10^{-2}. McMillen and Foster's[9], Abrams'[5], and Lefebvre du Prey's[8] experimental data for this capillary number correlation are shown in Fig. 1.

The theory of the relationship between the mechanics of individual blobs and the statistical flow behaviour of large populations of them is supplied, we believe, by percolation theory. We advance here a simple version which predicts the capillary number correlation just described. Before proceeding to it, a brief explanation of percolation theory is needed.

Percolation theory

Many transport processes can be successfully idealised as transport of an abstract fluid through an abstract medium. Diffusion of a solute through a solvent is an example: the fluid (solute) can be imagined to flow randomly, and the process is termed a diffusion process. If, on the other hand, the fluid flow paths are determined by the medium, but the medium itself is in some sense random, the process is called a percolation process. This classification and the introduction of percolation theory are due to Broadbent and Hammersley[10].

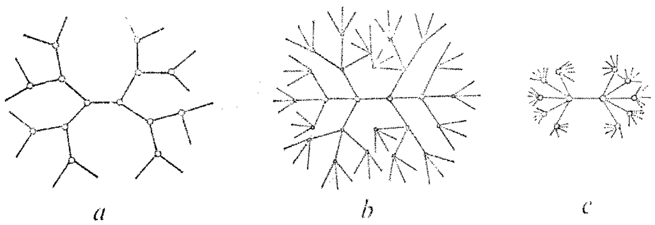


Fig. 3 Examples of Bethe lattices: $a, \sigma = 2$; $b, \sigma = 3$; $c, \sigma = 5$.

As a simple example, illustrating those features of percolation theory of relevance here, consider a very large, essentially infinite sheet of non-conducting material divided into identical square cells. At random, we replace a fraction of the cells containing the non-conductor with cells containing a conductor. If the fraction is small, most of the conducting cells occur as monomers—with no conducting cells as nearest neighbours. In particular, there is no continuous path of conducting cells long enough to span the sheet and, therefore, the sheet as a whole is non-conducting. As we increase the fraction of conducting cells, however, dimers, trimers, and larger clusters of connected conducting cells appear, until at some critical fraction, there appears an infinite cluster spanning the sheet. At this critical fraction, X_c, called the percolation threshold of the medium, the sheet becomes a conductor.

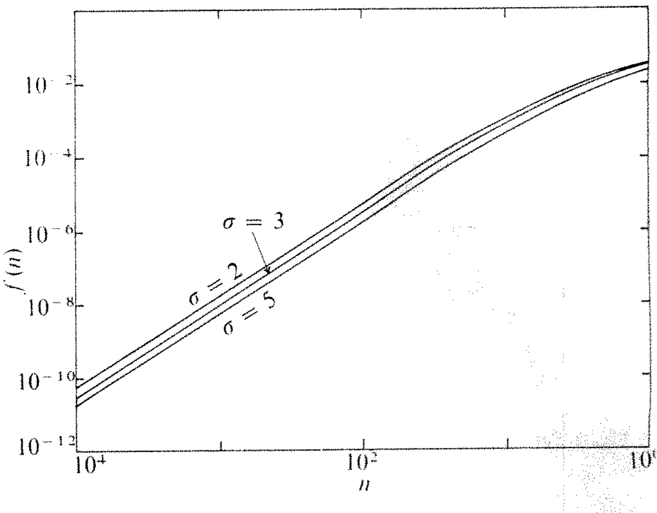


Fig. 4 Cluster size distribution function for a Bethe lattice.

Experiments with large two-dimensional (2-D) square arrays, such as the one described above, reveal an empirical X_c of 0.5 (ref. 11). That is, at least half the cells must be conducting, if the sheet as a whole is to show a non-zero conductivity. Three-dimensional (3-D) mixtures of conducting silver and non-conducting Bakelite show a conductivity threshold of 30 vol. % ($X_c = 0.3$)[12]. Small spheres of nickel mixed randomly with larger spheres of polyvinyl chloride and then compressed together to compaction show $X_c = 0.10$ (ref. 13). Computer studies of regular networks of chaotically distributed conductors and insulators yield percolation thresholds X_c ranging from 0.1 to 0.6, depending on the randomness of the distribution and connectivity of the network[11]. A computer-simulated 3-D continuum model gives $X_c = 0.29$ (ref. 14). Using percolation concepts, equation (9) which holds for an individual blob will now be used to derive a theoretical capillary number correlation.

Theory of residual phases

Imagine a one-dimensional 'waterflood', a process in which continuous non-wetting phase is being displaced from a porous medium by wetting phase. At a given time, the nonwetting phase saturation profile in the medium at some instant is represented by Fig. 2. At large Y (distance from position of wetting phase injection) the non-wetting phase is in the continuous regime, but at $Y = 0$, the non-wetting phase is in the blob regime, since only wetting phase is flowing. At some intermediate position, $Y = Y^*$, the non-wetting phase passes from the continuous to the blob regime. If the flow of non-wetting phase through the medium is such that at a given position Y, the nonwetting phase is distributed in a manner that leaves it randomly self-connected, then Y^* is the position where the non-wetting phase saturation, S_{nw} equals X_c, the percolation threshold for the void space of the solid matrix; that is, where S_{nw} is just low enough that the non-wetting phase no longer maintains long range continuity.

According to equation (9), if the capillary number of the flow is vanishingly small, as in very slow flow, then only long—essentially infinitely long—blobs will be mobile; that is, only that non-wetting phase which is in the continuous regime will flow. Thus, saturation must exceed $S_{nw} = X_c$ for non-wetting phase to flow if N_{Ca} is very small. Let waterflood residual non-wetting phase saturation be defined as

$$S_{nwrw} \equiv \lim_{N_{Ca}\to 0} S_{nwr}(N_{Ca})$$

then $S_{nwrw} = X_c$. Experimentally, as N_{Ca} becomes small, S_{nwr} asymptotically approaches S_{nwrw}, whose value typically lies between 0.2 and 0.5, as compared with a value of X_c of 0.25–0.30 for a completely random 3-D medium.

If, however, N_{Ca} is not infinitesimally small, then all blobs

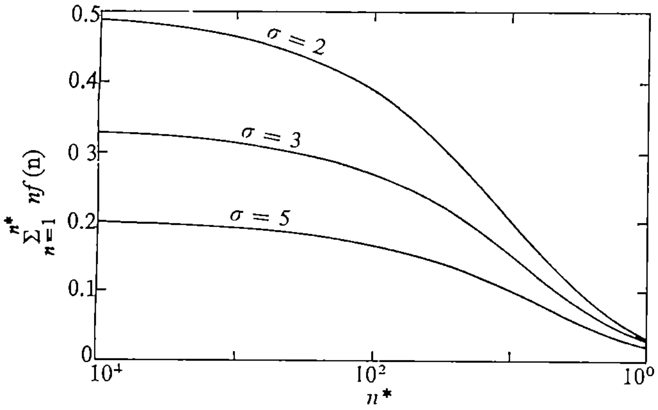


Fig. 5 Partial sum of the Bethe lattice cluster size distribution function.

of non-wetting phase longer than n^*, as given by equation (9), will flow, blobs shorter than n^* being trapped. When S_{nwr} decreases through $S_{\mathrm{nwr}} = X_{\mathrm{c}}$, the non-wetting phase breaks up into discontinuous blobs. Imagine that the distribution of projected lengths of these blobs is given; call it $f(n)$. That is, $f(n)$ is the expected number of blobs of length n in a large porous sample, divided by the total number of pores in the sample. The fraction of pore volume occupied by trapped blobs of length n is approximately $nf(n)$. (Here we ignore the difference between the number of pore bodies a blob occupies and its projected length, in pore diameters, along the direction of the pressure gradient.) On average, the volume of non-wetting phase trapped per pore volume, which is the residual non-wetting phase saturation, at a given N_{Ca} is

$$S_{\mathrm{nwr}} = \sum_{n=1}^{n^*} nf(n) \qquad (10)$$

where $n^* = \lambda\, N_{\mathrm{Ca}}{}^{-1} k_{\mathrm{rw}}\varphi$, as at (9).

If $f(n)$ were available a theoretical S_{nwr} (N_{Ca}) could be calculated from equations (9) and (10). If the already mentioned hypothesis is correct and the non-wetting phase is distributed in such a manner that it is randomly self-connected, then $f(n)$ may be obtained from a cluster size distribution for a 3-D random medium, a 2-D analogue of which was described above. A convenient approximation to such a medium, for which an analytical expression for the cluster size distribution is available, is the Bethe tree, or Bethe lattice. A Bethe lattice is a collection of bonds and sites, $\sigma+1$ bonds per site, illustrated in Fig. 3 for $\sigma = 2,3$, and 5. Let a fraction, X, of the bonds be conducting, the balance being non-conducting. Fischer and Essam[15] give an analytic expression for the size distribution of conductive clusters in a Bethe lattice; the distribution depends on σ and X. They find that the percolation threshold is at $X = X_{\mathrm{c}} = 1/\sigma$. In Fig. 1, the values of S_{nwrw}, and hence of X_{c}, can be seen to be about 0.5, 0.33, and 0.2, respectively, for Abrams' Berea sandstone, Foster and McMillen's Dalton sandstone, and Lefebvre du Prey's sintered teflon porous samples.

To model the topology of these porous media by Bethe lattices, one should take $\sigma = 2,3$, and 5, respectively, for the three media. The value of σ chosen to fit the value of S_{nwr} for a given porous medium is, in theory, a measure of the connectedness of the pore space of that medium. Higher values of σ indicate more inter-pore connections per pore, and thus a larger number of pathways one or more of which can be conductive to non-wetting phase.

With $X = X_{\mathrm{c}} = 1/\sigma$, $f(n)$ can be calculated from the analytic expression for Bethe trees in which $\sigma = 2,3$, and 5, respectively, with results shown in Fig. 4. Taking partial sums to get S_{nwr} as prescribed by equation (10) gives the results shown in Fig. 5.

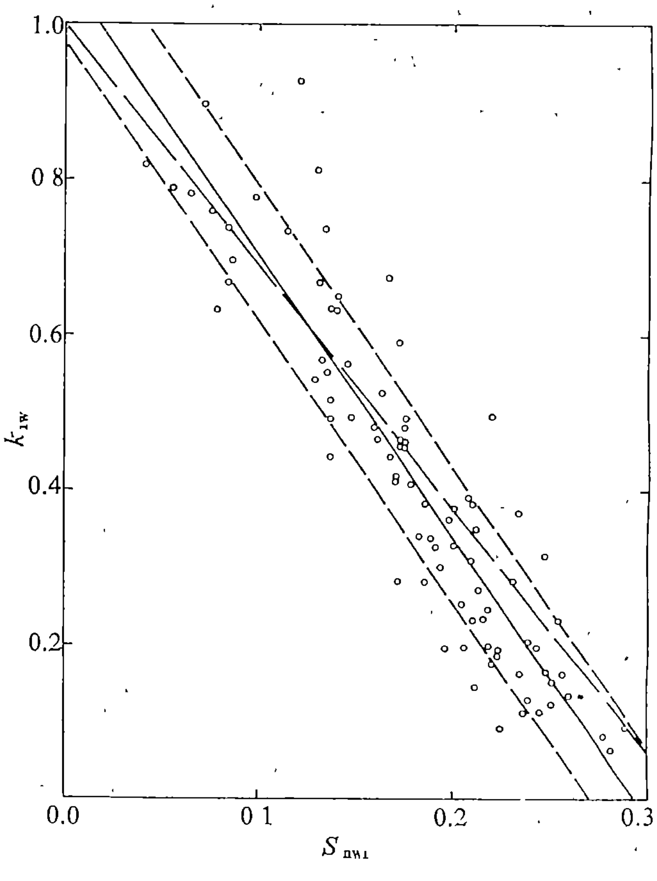
For his Dalton sandstone sample, Abrams[5] obtained for the relative permeability k_{rw} needed in equation (9), the experimental dependence on S_{nwr} shown in Fig. 6. This dependence is nearly linear over most of the range of S_{nwr}. By definition $k_{\mathrm{rw}} = 1$ at $S_{\mathrm{nwr}} = 0$, and experimentally $k_{\mathrm{rw}} = 0.06$ at $S_{\mathrm{nwr}} = S_{\mathrm{nwrw}}$ for Abrams' Dalton sandstone sample. A straight line between these two endpoints falls within a standard deviation of the least squares linear fit to the data (Fig. 6). Lacking the complete relation between k_{rw} and S_{nwr} for the other two porous media, we take as an estimate for all three media a straight line through the endpoints, the endpoint at $S_{\mathrm{nwr}} = S_{\mathrm{nwrw}}$ being provided by experiment for each porous medium.

For a given residual non-wetting phase saturation S_{nwr}, n^* can be found from $f(n)$ and equation (10), that is from Fig. 5; $k_{\mathrm{rw}}(S_{\mathrm{nwr}})$ can be found from the endpoint linear estimate; φ from experiment; and thus N_{Ca} can be calculated from equation (9) for a specified value of λ. There are two parameters: besides λ, which represents the relevant geometry of the pore space (introduced before equation (4) and at equation (7), there is behind $f(n)$ the topological parameter σ which we take as 2, 3 and 5, respectively, for the three porous media in Fig. 1. For λ we have tested various numbers: the value of 0.0065 based on Melrose and Brandner's estimates; and best-fit values of 0.0218 for Foster and McMillen's data, 0.0206 for Abrams' data, and 0.0487 for Lefebvre du Prey's data. The theoretical relations are plotted as S_{nwr} against N_{Ca} in Fig. 1*a–c*. The fitted λ's for the three consolidated porous media considered are consistently higher than the 0.0065 estimate for unconsolidated porous media. The difference is probably significant despite the uncertainties in Melrose and Brandner's estimates and the simplifying assumptions of the percolation theory employed here.

Conclusions

The relationship between residual non-wetting phase saturation and the capillary number of wetting phase flow in Fig. 1 is

Fig. 6 Relative permeability as a function of residual non-wetting phase saturation for Abram's Dalton sandstone sample. ———, Least squares linear fit; – – –, ± one standard deviation; — — —, endpoint linear approximation used in the theory.



successfully predicted from the mechanics of blob mobilisation and the percolation theory of transport. Central is a percolation concept of the size distribution of conductive clusters when conducting bonds are randomly located in a lattice. The clusters model the blobs of residual phase. Their size distribution depends on the connectivity properties of the lattice. These properties model the topology of the pore space. Geometry of the pore space dictates the relation between the size of a blob and critical flow conditions for mobilisation of that blob A practical implication of the theory is to reduce the problem of two-phase flow in porous media in first approximation to determining two parameters. One, the scale factor λ in this report, characterises the pore-geometry-dependent resistance to passage of a meniscus separating the phases. The other, the connectedness measure σ here, characterises the pore-topology-dependent number of alternate routes of meniscus passage. Concepts from percolation theory also lead to successful explanations of capillary pressure and relative permeability data from porous media[16,17].

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An 8,000-yr palaeoclimatic record of the 'Double-Hale' 45-yr solar cycle

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A series of isostatically-emerged Holocene beach ridges on the east side of Hudson Bay have been precisely surveyed and dated back to 8,300 BP. *They number 185 and show a nearly uniform rhythm of about 45 yr correlated here with the 'Double-Hale' solar magnetic cycle. (Time is in sidereal yr.) Longer correlations are suggested with eustatic sea-level curve and with planetary conjunction cycles. The coupling mechanism with terrestrial meteorological behaviour is believed to be by means of stratospheric generation of polar cirrus cloud and low-latitude ozone, affecting the planetary albedo.*

A CONTINUOUS sequence of 185 Holocene, radiocarbon-dated raised beach lines on the eastern side of Hudson Bay, Canada, have a 45±5-yr cyclicity that reflects storminess and high tide crescendos (Fig. 1). The strandlines have been precisely measured by theodolite surveys and followed by helicopter and air photograph studies along the coast. They can be observed as far as Ungava Bay (Hillaire-Marcel). They have been known since the seventeenth century but have only now been surveyed[1,2]. The beaches have been dated by radiocarbon analyses back from the present to 8,300 yr BP (sidereal) when the 'Tyrrell Sea' began to appear as a result of deglaciation during the Flandrian transgression. The relative uniformity of the 185 emerged beaches suggests a regular dynamic mechanism. Thus 8,300/185 = 44.86, conveniently rounded to 45. If the beach ages are plotted at 45-yr intervals, they are found to correspond closely to the radiocarbon dates.

A complicating factor is introduced by the unfortunate mid-Holocene departures of the radiocarbon chronology from sidereal time which tend to distort those ages to lower values, but these systematic errors are in part counteracted by comparable errors introduced by old carbon from freshwater dilution.

The uniformity of the 45±5-yr cyclicity is implied by the mean uniformity of beach widths, but their heights become progressively greater as one goes back in time because of an exponentially decaying isostatic curve. The present rate of uplift is 1.1 cm yr^{-1}, but around 7,000 to 8,000 BP it was ranging through 5–10 cm yr^{-1}, and is thus comparable with the behavior of the Baltic Shield in the Gulf of Bothnia[4].

The beaches are so sharp and clearly preserved due to a happy coincidence of genesis and history. Their materials were partly eroded and redeposited in ice-foot conditions, which is a much more energetic process than normal long-shore drift. Further, the continuous isostatic uplift rapidly takes each beach up above the reach of storm waves and wind-driven ice floes, so that they are preserved in their pristine state. The climate maintains a continuous permafrost beneath a sparse cover of arctic tundra vegetation. There is no human interference with their natural state.

A strandline of this sort is preserved in the topography only at certain moments in time, when, for example, $dI+dE\to 0$ where dI is isostatic uplift over a given interval, dt; and where dE is eustatic rise. When they are both in phase, and moving at the same rate, the shoreline will remain constant long enough for a recognisable beach to form. A second mechanism for beachridge formation is during cycles of increased storminess when seasonal onshore winds briefly imitate the effect of a eustatic rise.

Inasmuch as the isostatic factor has a very sluggish rate of velocity change (maximum 1 cm yr^{-1} in 100 yr), compared with eustatic factor ($\approx$ 1 cm yr^{-1} in 1–10 yr), any systematic differences between the isostatic uplift curve and the actual height of a given series of beach ridges must reflect an anomalous eustatic oscillation.

By constructing a glacioisostatic crustal recovery curve and carefully measuring the anomalous high and low series of beach levels, it was, therefore, possible to distinguish a eustatic factor from the isostatic, in the manner already demonstrated for Hudson Bay by Andrews[5]. Some of the early beaches are today uplifted to a maximum of 315 m.

Comparison of the eustatic residual with earlier versions of the Holocene eustatic curve obtained from low-latitude situations[5] shows an interesting degree of coincidence. It is clear that sudden drops or rise of sea level of the order of 2–5 m are worldwide in effect and may occur within time spans of 100–200 yr (dE = 1–5 cm yr^{-1}). These negative sea-level oscillations cor-

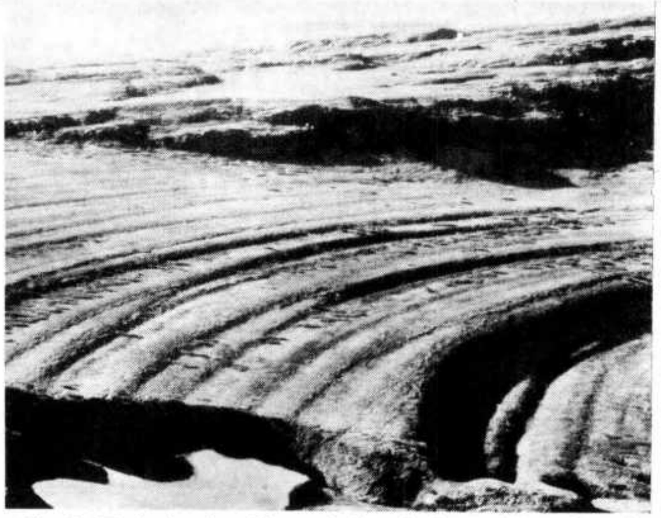

Fig. 1 Typical series of isostatically raised strandlines situated in Richmond Gulf on the eastern side of Hudson Bay, Canada. The sand-gravel beaches are well-preserved by permafrost and recur with a great regularity at around every 45 yr, representing cycles of storminess. A higher ridge, like that in the right foreground, represents a modification introduced by the eustatic factor, in this case a sharp regression followed by a rise, but not enough to overcome the isostatic emergence rate.

relate with documented climatic cooling and are assumed to be glacioeustatic (Fig. 2).

This fact can have a profound significance on climate prediction. If $dE = 1$ cm yr^{-1}, the equivalent ice volume is 4×10^3 km^3. The record shows that several cold spells sufficient to generate 1–2×10^6 km^3 of ice within one or two centuries have actually happened within the recent history of man in the present (Holocene) interglacial climatic conditions. The total ice volume of Greenland today is less than 2.6×10^6 km^3, equivalent to $dE\simeq6$m. Approximately 20 cold spells, each sufficient to generate at least 1×10^6 km^3 of excess glacier ice (above the global equilibrium level), have occurred during the last 6,000 yr, thus about one per 300 yr. No obviously predictable cyclicity is apparent.

The 45-yr cycle of storminess

The normal climate of the Hudson Bay is continental arctic, commonly rather calm and mild in summer and frigid in winter. Westerly storm tracks usually pass to the south. When, however, they periodically reach the latitude of Hudson Bay considerable wave action leads to rapid longshore drift and beach-building. The beach ridges were first attributed to these storms by Captain L. Foxe in 1631.

Because of the steady isostatic uplift (over 1 cm yr^{-1}) it is unlikely that after a 45-yr interval a new cycle of storms will cause the younger beach accumulation to overwhelm the earlier one. Thus the 185 beach lines preserved seem to represent a true record of somewhat regularly spaced meteorologic events. These are modulated, with larger and smaller beaches, by the glacio–eustatic factor mentioned above. A comparable but non-uplifted sequence of beach ridges on the Alaskan Arctic coast is attributed to meteorologic pulses related chronologically to the sunspot cycle[7].

Such a regular succession of climatically related geomorphic phenomena cannot be easily explained away by any steady state or stochastic hypothesis and invite a search for an exogenetic (cosmic) cause. The solar cycle seems to be the most obvious candidate. In recent years, it has been demonstrated with a reasonable degree of certainty that the sunspots are in some way expressions of solar climate that is tidally induced by planetary allignments[8]. If this is correct, two problems have to be considered: first, which of the planetary conjunctions or other configurations seem to have the leading role? And secondly, what is the mechanism of energy transfer from solar tidal behaviour to terrestrial climate?

If the second question is not met in some way the first does not become irrelevant, but in order to test the probability of the second, the timing of the first-named problem can possibly provide some guidance.

Solar tides

The timing of the basic '11-year cycle' is known to fluctuate between about 9 and 13 yr, but it is statistically around 11.1–11.3, thus close to that of the jovian yr, 11.8 sidereal yr. Equally, or more important, are the periods of other smaller but closer gravitational bodies affecting the Sun, notably Venus, Mercury and the Earth–Moon pair[8]. As with oceanic tides the problem of solar tides is quite complex.

The three-century record of closely observed sunspot behaviour is highly informative. Clearly the 22-yr heliomagnetic or Hale cycle is perhaps more significant than the familiar 11-yr pattern, and a study of the peak sunspot numbers (maxima) discloses a fourfold repetition of the 11-yr sequence, usually within a progressive rise to a crescendo every 45 yr (that is 40–49 yr, the so-called Double Hale cycle). Turning points (minima) were 1699, 1744, 1784, 1833, 1877, 1923, 1964 (ref. 9). A multiple (the King-Hele cycle) around 178–180 yr, gives the turning points in the long term sunspot series.

If one considers the less precise but historically quite well documented sunspot record of the Chinese[10] it becomes clear that there were lengthy periods with few sunspots (those observable without telescopes). The great sunspot dearth of the middle ages is particularly well known. In the record of planetary motions, the year AD 1433 is particularly significant, being the zero check year: with all planets in conjunction[11]. It is thus feasable to mark out various longer cycles from this date in order to see what terrestrial phenomena, if any, seem to match them[12]. The next significant planetary conjunction should be AD 1982.

Sidereal and radiocarbon years

Care should be taken to remember that the astronomical conjunctions are usually listed in terms of sidereal years, whereas much of the Holocene geological record is documented in radiocarbon years, which as mentioned earlier, have variable values. While at the beginning of the Holocene (10,000 yr BP), according to the varve-record, the departure was probably only of the order of ±200 yr, the middle Holocene was marked by errors in the radiocarbon age of 800 or even 1,000 yr, appeared too young[13,14].

The problem has to do with solar wind interacting with the earth's magnetic field which, as Bucha[15] has shown, has been weakening within the period of precision observation. This causes fewer cosmic rays to be deflected and thus more radiocarbon is generated. Accurately counted tree-ring samples indicate that between 200 and 700 BP there was a ^{14}C flux up to 2% above normal and thus dates in this period are up to 60 yr too young. Back to about 3,000 BP there were minor fluctuations, with some dates too old, but during the mid-Holocene there was a systematic departure giving dates too young by up to 800 yr (around 6,500 BP). The short term departures can also cause problems, because statistical peaks in site dates[15] may be artificially amplified; while the groupings almost certainly have a geomorphic–climatogenic cause, the possibility of ^{14}C flux variations should always be tested using tree-ring or varve standards, if possible.

An interrelationship between geomorphic–climatogenic events (such as eustasy, glaciation) and terrestrial magnetism is proposed by Olaussen and Svenonius[17]. It is significant that they correlate ice-loading and water-load distribution with reversals and predicted a magnetic reversal for some stage during the Flandrian transgression. A Gothenburg Event around 13,750–12,350 BP has, in fact, been widely recorded[18,19]. It could be expected that briefly during such a reversal there would be a regular deluge of radiocarbon over the whole Earth that for a few centuries might introduce seriously young dates.

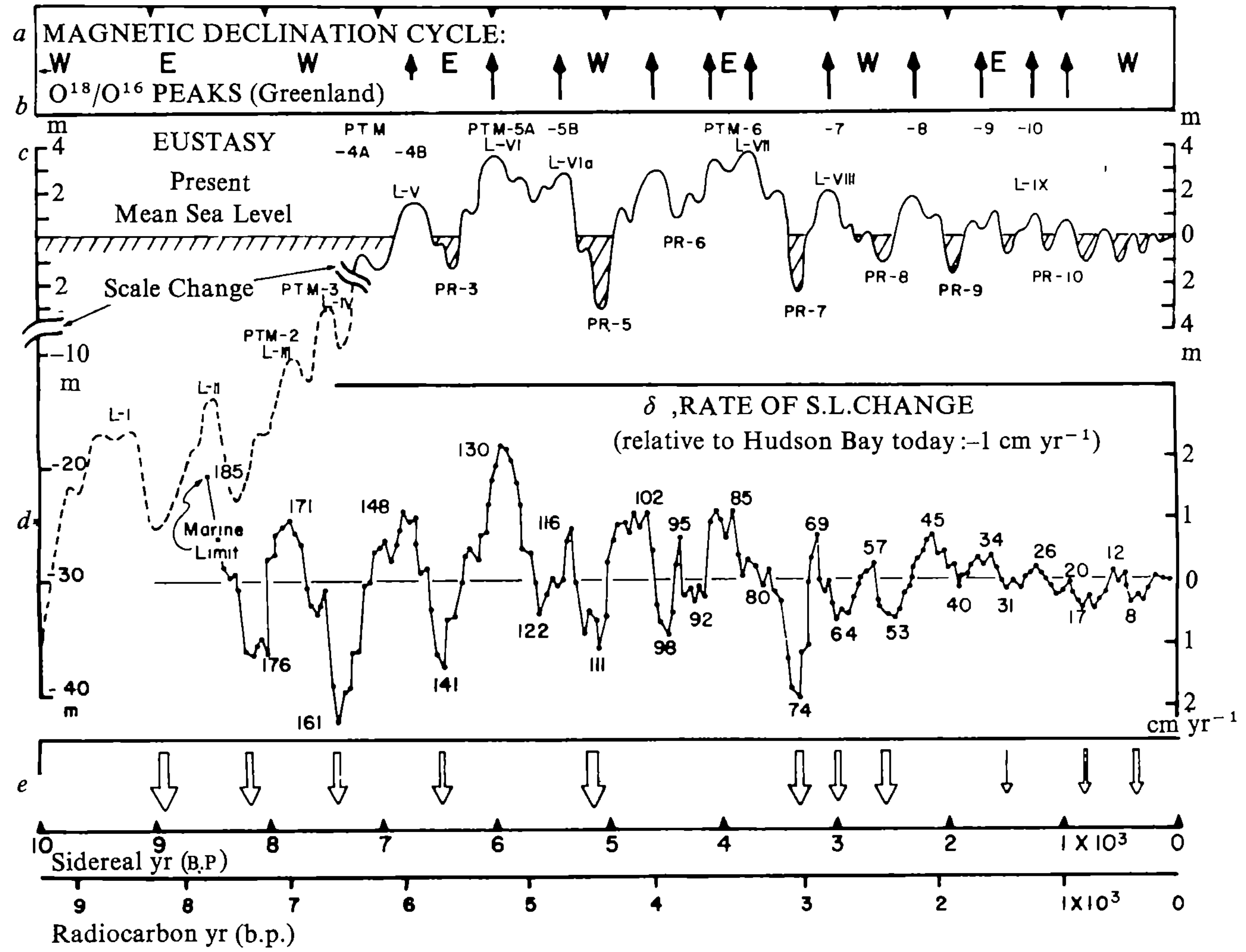

Fig. 2 Five records of 10,000 yr Holocene climate indicators compared. *a*, Magnetic declination cycle[33]: times of E–W inflections seem to represent times of lowest latitude (continental) sites for the magnetic pole, the present site being close to Hudson Bay, while the intermediate passage is over the ocean. (Question: does this have a climatic triggering effect?). *b*, The $^{18}O/^{16}O$ isotope peaks, as indicated by the Camp Century ice core from Greenland[35]. ^{18}O increases correspond to warmer fluctuations and to highest eustatic sea levels in the tropical oceans[36]. (Note: although there has been criticism and revisions of this record, the younger part shown here is not affected by the modifications and is apparently consistent for our own data shown below). *c*, Eustasy. This curve represents a slight updating of the Fairbridge Curve of 1961[30] and 1976[6], adjusted to sidereal time. Essentially, it represents a statistical summation of geomorphic sea-level traces (beach ridges, inter-tidal facies, coral in growth position) dated by radiocarbon. No adjustments are made for postglacial hydro-isostatic loading effects. PTM 1/10 peaks are based on Mörner's[36,37] Postglacial Transgression Maxima in S.W. Sweden. L-I/IX are Tooley's[38] Lytham raised beach levels from N.W. England. A comparable but younger series from New Zealand[39], and numerous other compatible records (Ters, Greensmith, Berglund, Martinsson), are omitted to avoid clutter. PR 1/10 are Mörner's Postglacial Regression Maxima. *d*, Delta, E, the rate of sea level change, as determined by Hillaire-Marcel[1,2] relative to the present-day east coast of Hudson Bay. Here the approximate crustal rise today is 1 cm yr^{-1} and the record, with the isostatic variability removed, now indicates rates of sea-level rise and fall. The inflection points (of no change) are numbered according to a continuous set of 185 numbered beach ridges on Hudson Bay, dating back 8,300 yr (sidereal). The relatively uniform geomorphic spacing of the beach ridges suggests a cyclicity of ~ 45 yr in storminess on Hudson Bay. *e*, Glacial advances. Major advances of mountain glaciers are known from radiocarbon dating of distal moraine belts, as recently summarised by Denton and Karlen[40] and others. Material is taken mainly from Alaska, Greenland, Scandinavia (Lapland), Patagonia and New Zealand: main advances occur simultaneously in both hemispheres. An almost identical set of dates was independently obtained at the same time from the Alpine glaciers by Patzelt[41]. Size of arrow is scaled to reflect magnitude of advance.

Little ice ages

It is not suggested that a regularly recurring minor planetary event of this sort can initiate an ice age condition. On the contrary, even the massive effects of the Milankovitch procession-tilt-orbital, now widely accepted[30–32], cannot generate glaciation phases on an earth that is not palaeogeographically prepared, which requires a blocked oceanic circulation and an asymmetric distribution of major continental land areas in one hemisphere, to create climatically sensitive latitudes. Only 10% of all geological time has thus been favourable to ice ages, and within an ice age of perhaps 2–10 × 10^{6} yr only 10% of that time is occupied by runaway glacial advances of continental dimensions (Pleniglacials). The rest of the time is filled by interglacial or stadial/interstadial climates. Nevertheless, it would only take a minor temperature departure for a few centuries in the north of Britain to trigger another glacial stage in the present sensitive state of global climatic instability. What is needed to explain little ice ages of a few centuries or less is, therefore, merely a small climatic pulse.

The critical year AD 1433 was first brought to attention by Pettersson[33] who believed that it correlated with catastrophic tides and flooding. In fact, the oceanic tidal effect of planetary conjunctions is quite minor, but the solar tidal response may have been quite different. This AD 1433 is the all-planetary conjunction, the so-called zero-check year. From Schove's long term Chinese sunspot evidence and other data, the year 1433 coincided by a remarkable high but short-lived period of sunspot activity, and after a brief but dramatic rise (to the > 150 spot level) there was a second sunspot dearth (Maunder Minimum) at the same time attended by a major rise in ^{14}C

flux (ref. 25 and P. E. Damon). Historical climatic analyses[26-28] show that 1433 initiated a time of great instability, culminating in the great medieval little ice age, with its attendant human suffering. It seems that the conjunction time coincided with a high sunspot state, which immediately followed by a low-spot condition indicates a minor glacial event.

Accordingly, taking AD 1433 as the base year, the major planet conjunctions have been traced back through the Holocene. Several years ago, a first attempt at this was made by Stacey[11], but at that time it was limited by interpretations and errors in the radiocarbon record. He attempted to correlate the planetary periods with the Fairbridge eustatic curve, assuming the fluctuations of the latter to represent (inversely) the rise and fall of glaciation. What has come out from the more recent studies of the Holocene eustatic record[30,31] is that the negative fluctuations that correlate with glacier advances and little ice age conditions are very brief events within a rather mild interglacial climatic pattern. The negative sea-level fluctuations are of the order of 3–5 m. In the last 8,000 yr there have been seven of these major departures; they show up equally in the tropical eustatic curve, in the Hudson Bay beach ridge curve and in numbers of glaciological and marine sedimentological curves. They are rather evenly spaced and despite difficulties in establishing their sidereal dates mentioned above, their regularity is striking. It was therefore considered that the seven recurrences of the 1,134-yr planetary conjunction stadial cycle should be seriously considered as being involved in providing a trigger mechanism.

With the reservations mentioned earlier concerning radiocarbon chronologies (apart from geological field errors) we would, nevertheless, like to draw attention to both the 567-yr and 1,134-yr astronomical cycles (major planets in conjunction). Going back from AD 1433 (= 517 BP), the turning points on the 1,134-yr cycle were 1,651, 2,785, 3,919, 5,053, 6,187 and 7,321 BP (sidereal) years. The hemicycle years (the 567-yr sequence) were 1,084, 2,218, 3,352, 4,486, 5,620, 6,754 and 7,888 BP.

Equally important in all probability is the solar activity tidal cycle (King-Hele) of 178–179 years. The Camp Century (Greenland) ice core showed both this and the 80-yr Gleissberg Cycle. It appears in the major Hudson Bay anomalies and various other records.

Certain coincidences are rather compelling: the great mid-Holocene glacial readvance (known in all glaciated regions), and identified in world oceans with a 6-m drop in sea level (PR-5 of the Baltic and beach 111 of Hudson Bay), coincides precisely with the fourth cycle back from AD 1433, that is, at 5,053 BP. It also matches the 25th cycle back of the 178–180-yr King-Hele cycle. Other dates are less than compelling. At present, we can only say the results look interesting.

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The size of Rous sarcoma virus mRNAs active in cell-free translation

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Sedimentation analysis of mRNA from RSV-infected chick cells suggests that Pr76, the precursor to the virus structural proteins, is synthesised on a mRNA of similar size to virion RNA, whereas a protein with molecular weight 70,000 tentatively identified as the non-glycosylated precursor to the viral glycoprotein, gp85, is synthesised on a smaller size class of mRNA.

ROUS sarcoma virus (RSV) contains a genomic virion 70S RNA which in denaturing conditions dissociates into two apparently identical 30–40S RNA subunits[1-5]. We and others have translated RSV virion 30–40S RNA in cell-free systems derived from mammalian cells[6,7]. The major *in vitro* product of RSV virion 30–40S RNA is a polypeptide similar, if not identical, to the 76,000 molecular weight precursor to the group-specific (gs) antigens, Pr76, which is found in RSV-infected cells[6-8]. Pr76 synthesised *in vitro* in response to RSV virion 30-40S RNA can be labelled with *N*-formyl-methionine derived from the initiator tRNA, fMet-tRNA_F. Since fMet can

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only be incorporated at the N terminus, this result indicates that the N-terminal end of Pr76 arises directly from an initiation event and not as a result of cleavage of a larger molecule. Since all other active initiation sites on eukaryotic mRNAs seem to be near the 5′ end of mRNA molecules, we interpret this result to mean that the gene for the gs antigens is located at the 5′ end of the viral RNA (ref. 6).

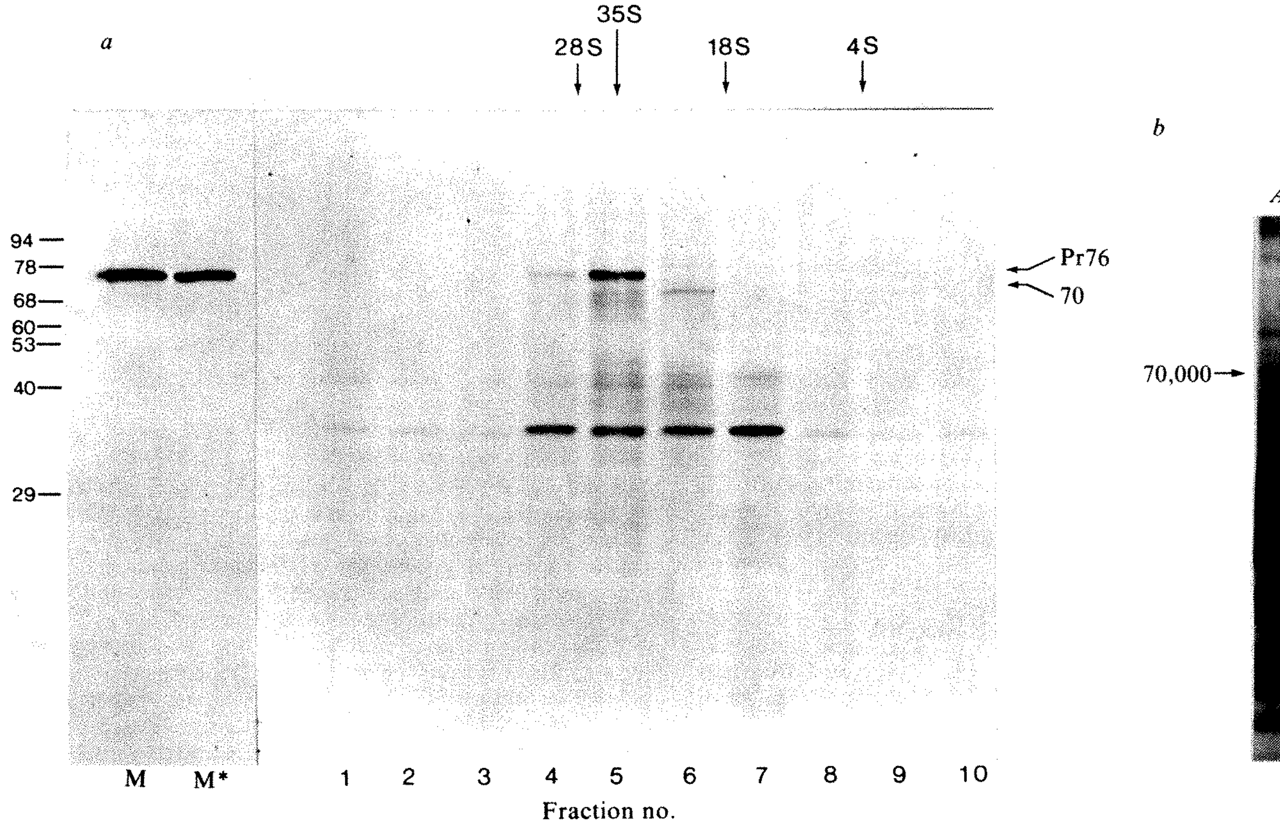
In addition to the gene for the gs antigens, the *gag* gene, RSV virion RNA contains genes for the viral reverse transcriptase, the *pol* gene[9]; the viral envelope proteins, the *env* gene[1,5]; and a gene coding for cell transformation, the *src* gene[1,4,5]. The *src* gene has been positioned at the 3′ end of the 30–40S RNA adjacent to the 3′ poly(A) tail, and the *env* gene to the 5′ side of the *src* gene[1,4,5]. Whether the *src* gene encodes a protein is not yet known.

All the virus-specific RNA present in RSV-infected cells has the same sense as the virion RNA (ref. 10), and two size classes of viral RNA, both of which are present in polyribosomes, have been found in the cytoplasm of RNA tumour virus-infected cells. These are 30–40S virion-like RNA, and a smaller RNA species described by several authors as desimenting between 14S and 27S, the precise size estimate varying with the virus examined and the techniques used[11–15].

These observations have led to various models for RNA tumour virus protein synthesis[6]. At one extreme is the suggestion that 30–40S RNA is the only viral mRNA, and that the viral proteins are synthesised as a single polypeptide precursor which is subsequently cleaved. At the other extreme is a model in which each viral gene is transcribed into a separate mRNA species. The first model would predict that equimolar amounts of each of the viral proteins are synthesised in infected cells, and would consider the sub-genomic viral RNAs and the failure to make anything other than Pr76 *in vitro* in response to 30–40S virion RNA as experimental artefacts. By contrast the second model predicts that since each mRNA may be present in a different amount and translated at a different efficiency, the viral proteins need not be synthesised in equimolar amounts. This model also suggests that virion 30–40S RNA has internal initiation sites and that they are inactive.

To examine the mechanism by which RNA tumour virus proteins are synthesised, we have isolated total poly(A)-containing cytoplasmic mRNA from RSV-infected chick cells. This mRNA has been fractionated on denaturing sucrose gradients, translated in mammalian cell-free systems derived from mouse ascites and L cells. Because infection by RNA tumour viruses does not shut off host-cell metabolism and viral

Fig. 1 *a*, SDS–polyacrylamide gel electrophoresis of viral polypeptides synthesised in the ascites cell-free system in response to fractionated RSV-infected chick embryo fibroblast mRNA and RSV virion RNA. Total poly(A)-containing RNA (mRNA) was isolated from c/o chick embryo fibroblasts infected with cloned nd RSV using methods described previously[6,16]. mRNA (25 μg) and ^{32}P-labelled whole mouse embryo non-poly(A)-containing cytoplasmic RNAs were fractionated separately on two 4.5-ml 2–10% sucrose gradients containing 85% formamide[17]. RNA samples were heated at 37 °C for 5 min in 85% formamide buffer before centrifugation for 16 h at 40,000 r.p.m. in a Beckman SW56 rotor at 25 °C. Gradients were fractionated and 30 μg carrier ascites cell tRNA was added to each fraction of the mRNA gradient. The RNA was precipitated after addition of 0.2 M NaCl and 2.5 vol ethanol, reprecipitated twice, washed once in 95% ethanol and dissolved in water. RSV virion 30–40S RNA was prepared as described[6]. RNA from the gradient fractions 1–10 and virion RNA (M) were translated in the mouse ascites cell-free system[6] and 20 μl samples of the cell-free products immunoprecipitated using the double antibody method[6] with antiserum raised against whole disrupted AMV. The products made in response to virion RNA were also immunoprecipitated with antiserum raised against AMV p12 (M*). The immunoprecipitates were analysed by electrophoresis on a single 15% SDS-polyacrylamide slab gel and the gel prepared for fluorography as described[18]. Because the counts incorporated in response to virion RNA were greater than with virion RNA, the exposure time of tracks M and M* was only 8 h whereas tracks 1–10 were exposed for 2 weeks. The positions of the marker RNAs run in the parallel gradient are indicated. *b*, SDS–polyacrylamide gel electrophoresis following immunoprecipitation with anti-AMV gp85 serum of polypeptides synthesised in the ascites cell-free system in response to 20–25S RNA from RSV-infected chick cells (*A*) and uninfected duck cell (*B*). mRNA was isolated from infected chick embryo fibroblasts and uninfected duck cells and fractionated on 85% formamide sucrose gradients as for *a*. RNA (referred to as 20S–25S RNA) sedimenting between the positions of the 18S and 28S rRNA markers run in a parallel gradient was pooled, precipitated and translated *in vitro*. Samples of the cell-free products were reacted with anti-AMV gp85 serum[19], immunoprecipitated and analysed on 15% SDS–polyacrylamide gels



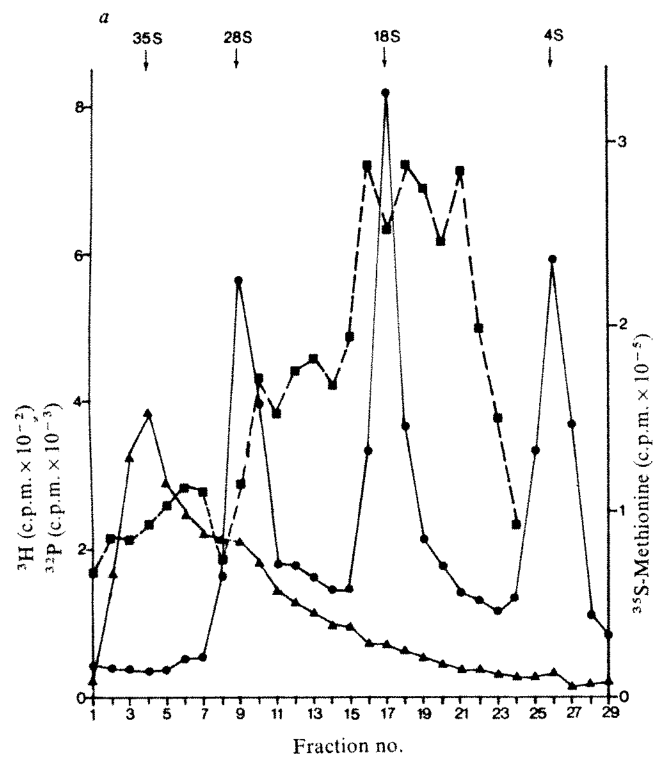
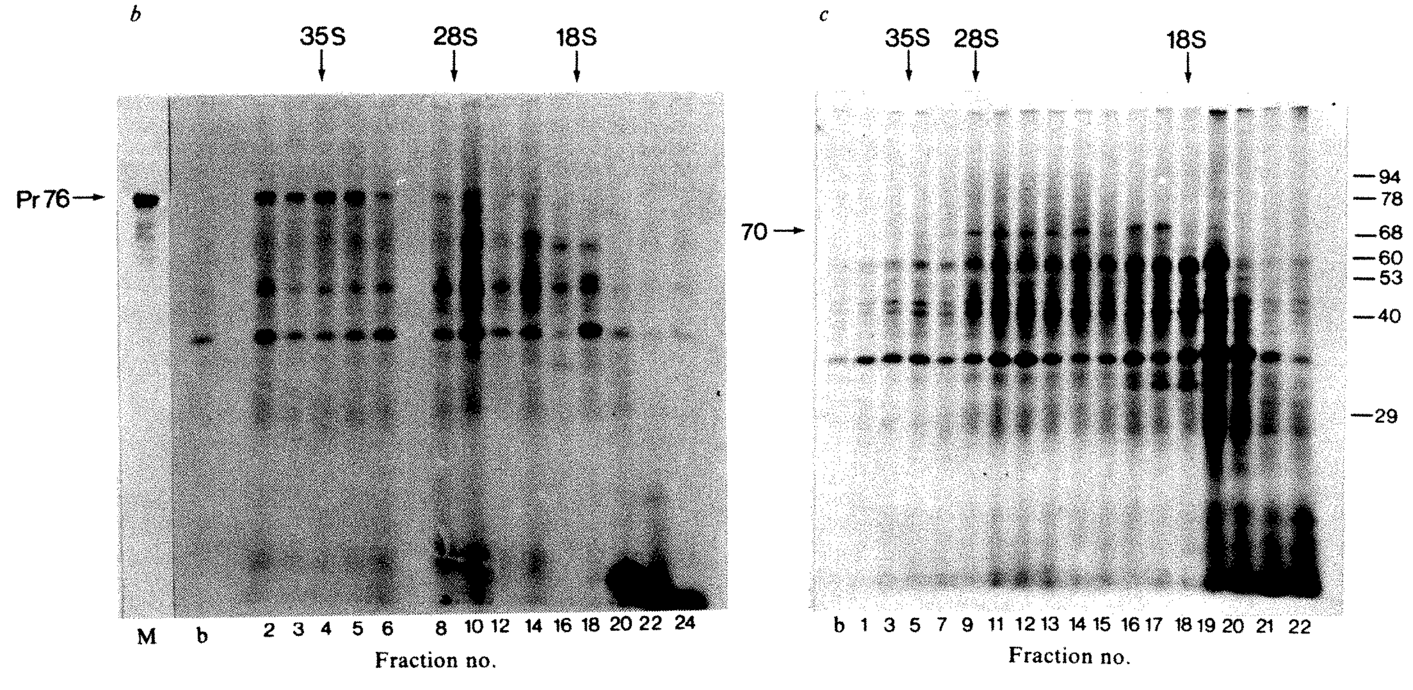


Fig. 2 *a*, Calibration of 5–20% sucrose gradient containing 50% formamide. Total RSV-infected chick embryo fibroblast mRNA (50 μg) was denatured at 37 °C for 5 min in 50% dimethylformamide, 25% dimethylsulphoxide and fractionated on a 50% formamide sucrose gradient[20]. Centrifugation was for 12 h at 35,000 r.p.m. in a Beckman SW40 rotor at 20 °C. ^{32}P-labelled non-poly (A)-containing cytoplasmic RNAs (●) and ^{3}H-labelled RSV 30–40S RNA (▲) were mixed, denatured and fractionated on a parallel gradient. mRNA was recovered from the gradient fractions as described in Fig. 1*a* and translated in an L-cell system supplemented with 50 μg crude rabbit reticulocyte initiation factors[21] per 50 μl incubation. The ^{35}S-methionine incorporated into TCA-insoluble material in a 2-μl sample of the cell-free system in response to RNA from the gradient fractions is, also indicated (■). *b* and *c*, SDS–polyacrylamide gel electrophoresis following immunoprecipitation with anti-AMV p12 serum and anti-AMV gp85 serum of the polypeptides synthesised in the L-cell system in response to RSV-infected chick cell mRNA fractionated on a 5–20% sucrose gradient containing 50% formamide. *b*, Samples (20 μl) of the products made in response to selected fractions of gradient fractionated mRNA (*a*) were reacted with 5 μl normal rabbit serum and incubated at 22 °C for 90 min. The rabbit IgG was removed by binding to protein A present on the surface of *Staphylococcus aureus*[22], followed by centrifugation. This procedure was designed to reduce the background of nonspecific precipitation. The supernatant was reacted with 5 μl rabbit anti-AMV p12 antiserum and the immunoprecipitates collected using the double antibody method[6]. An incubation containing no added RNA (b), and one containing virion 30–40S RNA (M) were treated in the same way. All the samples were run on the same 15% SDS–polyacrylamide gel and the gel was subjected to fluorography. Because of the differences in counts, track M was exposed for only 24 h whereas the remainder of the gel was exposed for 3 weeks. *c*, As for *b* except that immunoprecipitation was with anti-AMV gp85 serum and a different selection of fractions were translated.


b
35S
28S
18S
Pr76
M b 2 3 4 5 6 8 10 12 14 16 18 20 22 24
Fraction no.
c
35S 28S
18S
70
94
78
68
60
53
40
29
b 1 3 5 7 9 11 12 13 14 15 16 17 18 19 20 21 22
Fraction no.

mRNA constitutes only a small fraction of total mRNA activity, the viral proteins made *in vitro* were isolated by immunoprecipitation using various antisera raised against avian RNA tumour virus proteins.

Fractionation of infected cell mRNA

Poly(A)-containing cytoplasmic RNA was isolated from c/o chick embryo fibroblasts transformed with non-defective Prague-C RNA, from uninfected chick cells, and from duck fibroblasts (which do not contain detectable endogenous viral sequences). These mRNAs were fractionated on 2–10% sucrose gradients containing 85% formamide. ^{32}P-labelled 18S and 28S rRNA and ^{3}H-labelled RSV virion 30–40S RNA were sedimented as markers in a parallel gradient. RNA from each of the gradient fractions was recovered and translated in the ascites cell-free system. The cell-free products were immunoprecipitated with antiserum raised against detergent-disrupted AMV, which contains antibodies against both the gs antigens and the envelope glycoproteins, and the immunoprecipitates were analysed by electrophoresis on SDS–polyacrylamide slab gels. RSV virion 30–40S RNA was translated, immunoprecipitated and electrophoresed together with the products made in response to fractionated mRNA.

Figure 1*a* shows that only one polypeptide is immunoprecipitated from cell-free incubations containing added virion

30–40S RNA, using either anti-AMV or anti-p12 sera. We have previously identified this as Pr76, the precursor to the viral structural proteins[6]. Because less radioactivity is present in the immunoprecipitates of material made in response to the fractionated mRNA, this region of the gel was autoradiographed for longer. This reveals that a background of proteins (notably actin) which is present in the material immunoprecipitated from all fractions, is also immunoprecipitated by control serum, and uninfected duck cell mRNA products. In addition to the background, there is a polypeptide which comigrates with marker Pr76 and is synthesised from an mRNA activity from RSV-infected cells which sediments slightly slower than the 28S RNA marker (Fig. 1*a*). On 85% formamide sucrose gradients, virion 30–40S RNA also sediments slower than 28S RNA.

A further polypeptide which we estimate to have an apparent molecular weight of 70,000 is immunoprecipitated by antiserum to disrupted virions from the products made in response to mRNA from infected cells. The mRNA coding for the 70,000 polypeptide sediments slower than the mRNA coding for putative Pr76. Virion RNA does not code for the 70,000 protein (Fig. 1*a* track M).

The 70,000 molecular weight polypeptide synthesised by RSV-infected cell mRNA is not immunoprecipitated by control rabbit serum, nor is it detected when uninfected chick or duck cell mRNA are translated and immunoprecipitated (data not shown). To characterise the 70,000 protein further we have used antisera raised against purified viral components. The 70,000-MW polypeptide was not immunoprecipitated by antiserum raised specifically against the group-specific antigen, P12, which precipitates Pr76 made *in vitro*[6]. Antiserum raised specifically against the major viral glycoprotein gp85, however, immunoprecipitated the 70,000 protein from the translation products of total unfractionated infected cell mRNA and of the 20–25S fraction. Figure 1*b* shows the immunoprecipitates obtained with anti-gp85 from the products made in response to 20–25S RNA from infected chick cells and uninfected duck cells. Although there is a high background it is clear that the 70,000 protein is only present in the product made by infected cell mRNA. The results of the immunoprecipitation experiments indicate that the 70,000 polypeptide may be a virus-specific molecule, and if so, that it may be related to the envelope glycoprotein gp85.

The experiment shown in Fig. 1 used 85% formamide sucrose gradients because they are reported to be highly denaturing[17] and we have previously found such conditions to be necessary to reduce mRNA–mRNA interactions to a minimum[16]. These gradients, however, do suffer the disadvantage that mRNAs larger than about 18S do not sediment at their true rate. Indeed, as already mentioned, ^{3}H-labelled 30–40S virion RNA sediments slower than 28S rRNA in these conditions. Thus, although the results presented in Fig. 1 do not allow us to assign accurate sedimentation constants to the different RNA fractions, they do indicate that the mRNA coding for putative Pr76 is different and separable from that coding for the 70,000-dalton protein.

To achieve a greater separation of the two mRNAs from RSV-infected cells, and a more accurate estimate of their size, we repeated the experiment but in this case used 50% formamide sucrose gradients and collected more fractions. To identify the products we translated RNA from selected fractions in an L-cell S30 and immunoprecipitated the products with the two specific antisera, against the purified gs antigen P12 and against purified viral glycoprotein gp85, mentioned above. To reduce the background of immunoprecipitated material which we found to be higher when using the monospecific antisera (Fig. 1*b*) we introduced a pre-immunoprecipitation step using control serum as described in the figure legend.

Figure 2*a* shows the position of 18S and 28S rRNA and 30–40S virion RNA sedimented on a 5–20% sucrose gradient containing 50% formamide, and also the ^{35}S-methionine incorporation into TCA-insoluble material when infected cell mRNA was fractionated on a parallel gradient and translated in the L-cell system. Figure 2*b* shows that a polypeptide, which comigrates on SDS–polyacrylamide gel electrophoresis with marker Pr76, is immunoprecipitated by anti-P12 serum from the products made in respones to an mRNA activity which cosediments with virion 30–40S RNA. Pr76 cannot be detected in the products made from mRNA sedimenting slower than about 28S.

When the translation products of infected cell mRNA in the L-cell system are subjected to immunoprecipitation with antiserum raised against AMV gp85, Pr76 is not detected in the immunoprecipitates (Fig. 2*c*). The 70,000-MW polypeptide can be seen, however, and is translated from an mRNA activity well separated from that coding for Pr76, sedimenting between about 20 and 28S. In spite of the pre-immunoprecipitation step, a considerable background still remains in the immunoprecipitates with anti-gp85 serum (Fig. 2*c*). Nevertheless immunoprecipitation of the 70,000 protein seems to be specific since when uninfected duck cell mRNA was translated and immunoprecipitated with the anti-gp85 serum, the background was still seen but the 70,000 protein was absent (for example see Fig. 1*b*); further, the 70,000 protein was not immunoprecipitated by the anti-P12 serum (Fig. 2*b*).

A model for the translation of viral RNA

The data presented here suggest that in chick cells productively infected with non-defective RSV, the only RNA species which can actively direct the synthesis of the gs antigen precursor Pr76 is a polyadenylated RNA of the same size as virion 30–40S RNA. Whether the 30–40S cellular RNA species which codes *in vitro* for the synthesis of Pr76 is identical to virion 30–40S RNA, or whether there are two forms of viral RNA of this size, one of which is translated and the other is encapsidated, is unknown. Although we have not yet measured the amount of viral RNA present in the infected-cell mRNA, estimates based on previously published reports[11,23–27] indicate that there is no striking difference in the translational efficiency of cellular 30–40S RNA and virion 30–40S RNA in coding for Pr76. Some of the high molecular weight viral RNA from infected cells may originate from nucleocapsid where *in vivo* it is probably not available for translation. If all the 30–40S RNA were encapsidated, however, and there was a virus-specific mRNA smaller than 30–40S RNA directing the synthesis of Pr76 in these cells, we should have detected it.

In addition to the Pr76 viral polypeptide, a 70,000-molecular weight polypeptide has been detected in the translation products of mRNA from infected cells. The suggestion that this polypeptide is a viral-specific product and possible envelope glycoprotein precursor is based on the finding that it is immunoprecipitated by anti-disrupted virus serum (Fig. 1*a*) and by anti-gp85 serum (Fig. 2*c*), and not by control or anti-P12 serum (Fig. 2*b*). Further, it cannot be detected in the translation products of uninfected cell mRNA (Fig. 1*b*).

We are not aware of any example of a eukaryotic protein that, following synthesis *in vitro*, is extensively glycosylated. It is therefore not surprising that the protein made *in vitro* does not migrate with the reported mobility of viral gp85. Our results are consistent, however, with the observation that when RSV-infected cells are pulse labelled and cell lysates subjected to immunoprecipitation with anti-gp85 serum, an incompletely or non-glycosylated precursor to gp85 is detected (P70) and this has an apparent molecular weight on SDS–polyacrylamide gels of 70,000 (ref. 28). Since the antiserum used was from the same laboratory as that used in our study and the apparent molecular weights of the two proteins are similar it seems possible that our 70,000 protein is the non-glycosylated polypeptide moiety of gp85. It must be emphasised, however, that the identification of the 70,000-molecular weight polypeptide translated from infected cell mRNA is based solely on immunoprecipitation. Rigorous identification of the polypeptide must await the development of new methods which will enable us to purify sufficient virus-specific cytoplasmic mRNA to give

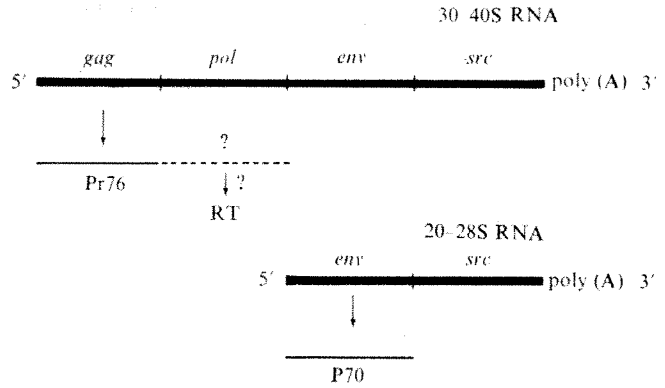


Fig. 3 Possible model for the synthesis of virion RNA proteins.

cell-free products which can be analysed by tryptic peptide fingerprinting.

The results presented here suggest that the two size classes of viral RNA that have been detected in the polysomes of infected cells by several groups both actively direct the synthesis of viral proteins. The finding that the mRNA activity sedimenting between 20–28S codes *in vitro* for a 70,000 polypeptide, but that virion RNA or virion-sized mRNA from infected cells do not code for this protein suggests that the two mRNAs have different coding activities. Possibly the 20–28S RNA represents the 3′ half of virion 30–40S RNA and the corresponding internal initiation sites for protein synthesis present in virion RNA is inactive (see Fig. 3). Such a model is similar to those already presented for the synthesis of Semliki forest virus structural proteins[29], polyoma virus capsid proteins[16], and several plant virus capsid proteins. Bloemendal and colleagues[34,35] have reported similar experiments to those described here and have shown that different species of viral mRNA from cells infected with murine leukaemia virus (MuLV) have different coding activities. These experiments are also consistent with the model shown in Fig. 3.

How the *pol* gene is translated is still unclear. Arlinghaus *et al.*[30] have reported that a precursor to the viral proteins of molecular weight 200,000 is present in MuLV-infected cells and that this includes the structural proteins and possibly the reverse transcriptase. Kerr *et al.*[31] have synthesised a 180,000-molecular weight polypeptide *in vitro* in response to MuLV 30–40S virion RNA. This protein includes the tryptic peptides of the structural proteins, but no further components have yet been identified. It is likely that the high molecular weight protein found in the murine system is the product of read-through from the *gag* gene into the *pol* gene, but that this readthrough does not occur in every case. Such a model would be similar to that for the synthesis of bacteriophage Qβ coat protein and its readthrough product, the A_1 protein[32] and for the synthesis of Mengo virus proteins late in infection[33].

While this manuscript was in preparation, Mueller-Lantzsch and Fan[36] reported that the only viral RNA species present in polysomes immunoprecipitated by anti-MuLV p30 serum from total infected cell polysomes has a sedimentation rate of 30–35S.

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Note added in proof: Since this manuscript was submitted other authors have reported that RSV subgenomic mRNAs from infected cells direct the synthesis of the viral glycoprotein when injected into chick cells[37] and oocytes[38].

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letters to nature

Cyg X-3: γ rays from a magnetic accretion disk

THE recent observation[1] of ~ 100-MeV γ rays from Cygnus X-3, which have a comparable luminosity to the X-ray emission and which vary in phase with the 4.8-h period displayed by the X-ray and infrared emission, has been taken[2, 3] as support for the suggestion[4] that the X-ray emission is produced by a very young pulsar in a short period binary system. In this letter, we show that the observed γ-ray luminosity can be produced by an accretion disk around a non-magnetic neutron star or low-mass black hole, in a magnetised corona which accelerates relativistic electrons that decay by inverse Compton losses modified through the photon–photon and pair-annihilation processes.

The dominant dissipative mechanism in the disk is assumed to be due to magnetic fields. The shearing motion of the disk increases the azimuthal field strength in the disk until the magnetic pressure is comparable with thermal (or radiation) pressures inside the disk. We then expect the field lines to bow apart from the disk to form a magnetic corona above the disk as described by Parker[5]. This process may also turn sufficient of the azimuthal field into poloidal directions so that a dynamo mechanism operates. Since the Alfvén speed in the corona exceeds that in the disk (where most of the matter resides) and since the lengthscales associated with field reversals should be comparable in both regions, we expect most of the dissipation of magnetic energy (through field annihilation and particle acceleration) to take place in the corona and not in the disk itself. There is a self-consistent equilibrium situation in which the disk is kept at X-ray temperatures producing photons with energy $E_x \sim 10$ keV. The relativistic electrons (energy $= \gamma m_e c^2$) generated by the field annihilation, lose energy predominantly by inverse Compton losses by upscattering the X-ray photons to γ-ray energies. Taking an X-ray luminosity[6] of $L_x \sim 5 \times 10^{37}$ erg s^{-1} to be coming from the inner part of a disk, a region of radius $R \sim 3 \times 10^6$ cm, we find that the energy density of X-ray photons is $\varepsilon_x \sim 3 \times 10^{13}$ erg cm^{-3} with corresponding X-ray photon number density $n_x \sim 2 \times 10^{21}$ cm^{-3}. For these parameters, and for $\gamma_2 = 10^{-2} \gamma \leqslant 1$, the energy loss timescale for an electron to inverse Compton scattering is $t_{ic} \sim 10^{-8} \gamma_2^{-1}$ and the corresponding radiation path length is $\lambda_{ic} \sim c t_{ic} \sim 3 \times 10^2 \ \gamma_2^{-1} \ll R$ (for standard disk densities[7], energy loss in the rough nuclear collisions is negligible). For synchrotron losses to be unimportant the magnetic field strength must satisfy $B^2/8\pi \leqslant \varepsilon_x$, that is $B \ll 3 \times 10^7$ G. The equipartition field in a standard accretion disk[7] at the above radius is $\leqslant 4 \times 10^7$ G. We may expect the magnetic field to be somewhat less in the corona and, hence, take $B = 10^7$ G.

To produce the required γ-ray photons with energy $E_\gamma \sim 100$ MeV we require $\gamma^2 \sim E_\gamma/E_x$, or $\gamma_2 \sim 1$. When $\gamma > m_e c^2/E_x \sim 50$, the Klein–Nishina cross section is relevant and the energy of the γ ray produced in a collision is $E_\gamma \sim \gamma m_e c^2$. The energy loss timescale for an electron with $\gamma_2 \gg 1$ is $t_{ic}^* \sim t_{ic}\gamma_2^2$.

Assuming that magnetic field reconnection takes place at a velocity $\sim 0.1 \ V_A$, where V_A is the Alfvén velocity in the corona[8], we may estimate the induced electric field to be $E \sim (0.1 V_A/C)B$. The particle density in the corona is sufficiently low that we may take $V_A \sim c$ and estimate $E \sim 10^6$ units. If the particle acceleration occurs over a length, λ cm, we have that $\gamma_2 \sim 10^{-3} eE\lambda/(m_e c^2) \sim 5\lambda$ (ref. 9). For self-consistency we demand that $\lambda \leqslant \lambda_{ic}$ and hence that $\gamma_2 \leqslant 0$. It seems possible, therefore, that the initial photons produced have energies $\gg 100$ MeV. We note, however, that photons with energies $E_\gamma \gtrsim E_s \sim 4(m_e c^2)^2/E_x \sim 100$ MeV) can undergo photon–photon interactions[10], with cross section $\sim \sigma_T$, the Thomson cross section, to produce electron–positron pairs with $\gamma_2 \geqslant 1$. The optical depth of such γ rays in our region is $\tau_\gamma \sim n_x \sigma_T R \sim 10^3$. The pairs so produced then lose their energy by inverse Compton interactions (on a timescale $\sim t_{ic}^* \sim \gamma_2/(n_x \sigma_T c)$ at a rate faster than they annihilate (on a timescale $\sim 10^2 \gamma_2/n_\pm \sigma_T c$ where $n_\pm$ is the electron–positron number density[11]). (We shall show that $n_\pm \sim n_\gamma$ and since $L_x \sim L_\gamma$ then $n_x \sim 10^4 n_\gamma$). In this way, all photons are degraded to energies $\sim E_s$ before escaping from the corona. This also implies that the rate of formation of electron–positron pairs is comparable to the rate of production of ~ 100-MeV photons. The timescale for escape of photons is $t_p \sim R/c \sim 10^{-4}$ s whereas the annihilation timescale (now with $\gamma \sim 1$) is $t_\pm \sim 1/(n_\pm \sigma_T c)$. If n_γ is the number density of γ-ray photons with $E_\gamma \sim 100$ MeV, therefore, in a steady state $n_\pm/n_\gamma \sim t_\pm/\mathrm{t}_p$ and hence $n^2_\pm \sim n_\gamma/(R\sigma_T)$. Assuming a γ-ray luminosity $L_\gamma \sim 10^{37}$ erg s^{-1} we find that $n_\gamma \sim 10^{17}$ cm^{-3} and hence that $n_\pm \sim 2 \times 10^{17}$ cm^{-3}. The annihilation of the electron–positron pairs gives rise to a line in the region of 500 keV which contains a photon flux comparable with that in γ rays, that is a luminosity $L_\pm \sim (m_e c^2/E_\gamma) L_\gamma \sim 10^{35}$ erg s^{-1}.

Once the γ rays have been sufficiently degraded they can escape from the coronal region. About a half of them can escape to infinity but the rest enter the accretion disk itself. From the standard disk model we find the surface density of the disk to be ~ 5 g cm^{-3}, so that the γ rays are absorbed by the disk. They heat the disk to X-ray temperatures (thus ensuring the self-consistency of our assumption). In this model we find, therefore, that the rough equality of L_x and L_δ arises naturally.

We can further examine why this process produces γ rays for a disk model of Cyg X-3, but may not for other sources in which disks may be present. From the standard disk model, the magnetic field strength in the disk in the neighbourhood of the central regions is $\sim 10^8 (M/M_\odot)^{-\frac{1}{2}} (R/3R_s)^{-\frac{3}{4}}$ G (where M is the mass of the central object and $R_s = 2GMc^{-2}$) independent of the accretion rate. If, therefore, ε_x falls much below the value we find that for Cyg X-3 the dominant loss process for the relativistic particles is synchrotron radiation which produces photons in the X-ray range. Thus, if the luminosity of the X-ray source is too low, or if the area of the emitting region is too high (for example, for a $10 M_\odot$ black hole in Cyg X-1), the energy is radiated almost entirely as X rays, some thermally from the disk and some non-thermally as synchrotron radiation from the corona.

An irregular time variability, or the occasional disappearance of the γ-ray emission of Cyg X-3, could occur if $\dot{M}$ (and consequently ε_x) drops below the value required for inverse Compton losses to dominate over synchrotron, or if $\dot{M}$ increases to a value where the total luminosity approaches the Eddington value and disk symmetry is destroyed. In both cases, one could expect the γ-ray emission to dissappear while the X-ray emission remains, albeit with somewhat different spectral characteristics.

The 4.8-h variation in the observed γ-ray flux can be produced in the same manner as the X-ray variation, using the model of Pringle[12]. In this model, the X-ray variation is produced by the variation with binary phase of the electron scattering optical depth to the X-ray source through a dense wind emanating from the companion star. We expect the γ-ray variation to be more pronounced, since the X-ray variability is smoothed somewhat by the backscatting of X-ray photons. On the other hand, backscattered γ-ray photons are degraded by Compton recoil.

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Are striations on Phobos evidence for tidal stress?

VIKING orbiter photographs of Phobos, the inner satellite of Mars, reveal a curious series of striations or grooves over much of the surface[1]. These features cross over large eroded craters but are in turn interrupted by very small well defined craters, which indicate an origin substantially later than the time of formation of the satellite itself when the large scale cratering presumably occurred. The grooves have no obvious connection with the 'crater chains' also seen in Phobos, and their nearly parallel spacing seems to rule out an origin due to primary or secondary impacts. We suggest that some of these features are related to readjustment of the satellite's figure with increasing tidal stress as the orbit evolves inwards under the action of tidal friction.

The present rate of orbital decay[2] suggests that Phobos's original orbit had a radius $a \sim 5R$ (R is martian radius) and that the present orbit, at $2.76R$, will decay to the martian surface (unless the satellite breaks up first) within $\sim 10^8$ yr. The tidal (plus centrifugal) stress, $\sigma \propto a^{-3}$, has already increased by a factor of ~6 since the origin of Phobos.

The magnitude of σ is now of order $\Omega^2 \rho r^2 \approx 10^5$ dyn cm^{-2}, where Ω is the angular velocity, ρ the density, and r the radius of the satellite. If Phobos has a deep regolith layer of material similar to lunar dust, one might expect a material strength[3] near the surface as low as 10^3–10^4 dyn cm^{-2}. Thus even if the interior were perfectly rigid, tensile stress fields in the weakly cohesive surface regolith might give rise to patterns of parallel cracks. With the addition of a mechanism for the partial readjustment in the figure of Phobos under tidal stress, the surface cracks could then be widened into the observed series of grooves or graben.

For example, slow viscous creep due to subsurface permafrost could allow tensile cracks to widen. Spacecraft perturbations indicate a density[4] for Phobos of about 2 g cm^{-3}. This low value, together with the satellite's observed photometric and polarimetric properties[5], suggests a carbonaceous chondritic and hence water-rich composition, so that permafrost is not unreasonable. Shock waves due to impacting bodies might also enhance the readjustment of the figure.

If Phobos is homogeneous, its low mean density places it inside the classical Roche limit for the gravitational instability of a fluid body (ref. 6). Therefore, either the satellite has a denser core (we thank Dr S. Dermott for clarifying this point) or, if homogeneous, it possesses sufficient rigidity to prevent full readjustment on the timescale of orbital evolution ($\sim 10^8$ yr).

One very prominent series of grooves is arranged nearly concentrically around the tidal equator (normal to the satellite's long axis), which orientation is to be expected from tidal stress theory[7]. Shear failure at moderate depths, where the principal stresses are all compressive, might give rise in addition to transcurrent faults, and there is perhaps an indication of this in some of the Phobos pictures showing sets of obliquely intersecting striations.

If the width of the grooves is in fact due to tidal readjustments of the figure of Phobos, then the older craters should be systematically deformed from their initial nearly circular shapes. Craters near the tidal equator would be elongated parallel to the satellite's long axis. A similar suggestion was made by Öpik[8] with respect to the Moon, but the effect for Phobos would be more pronounced.

Striations and other deformations due to tidal stress should be nearly absent on Deimos. The outer satellite has always been beyond the synchronous distance ($6.0R$) and at present ($a = 6.92R$) has a tidal stress about 60 times weaker than that for Phobos.

An investigation is being made of the possible role of subsurface ice in producing the detailed morphology and distribution of tidal stress features on Phobos.

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Geomagnetic storms and electric fields in the equatorial ionosphere

LARGE variations of the geomagnetic field observed at ground level during a geomagnetic storm are generally attributed to various magnetospheric changes following the arrival of the solar plasma ejected during the solar flare. Using the direct measurements of equatorial electric field during a geomagnetic storm, it is shown here that the large decrease in the field observed near the dip equator is due to the reversal of the equatorial electrojet current. This event is caused by the imposition of an additional westward electric field on the equatorial ionosphere which was originated by the interaction of solar wind with the interplanetary magnetic field.

A standard type of geomagnetic storm consists of a sudden increase of the horizontal (H) field at ground level within a few minutes at all world stations (called storm sudden commencement—SSC) followed by the initial phase (IP) for a few hours when the H field is above normal. The main phase (MP) of the storm is supposed to start when the H field decreases below the pre-SSC level and ends when **H** reaches the minimum value. The recovery phase follows the main phase and may last one or two days. The history of a particular storm can be identified better by the temporal changes of the equatorial $\mathbf{D}_{st}$(H) values rather than from temporal variation of **H** at a particular station.

The first explanation of the various effects of the geomagnetic storm was by Chapman and Ferraro[1] with the concept of neutral ionisation moving from the Sun through the geomagnetic cavity. The SSC was explained in terms of an enhancement of the electric current induced on the surface of the cavity by the ion stream, and the MP was caused by a ring current surrounding the Earth. Dessler[2] suggested the SSC to be produced by hydromagnetic shock wave generated at the frontal surface. Rastogi and Trivedi[3] showed that the amplitude of SSC(H) at low latitudes is greatly enhanced in a way similar to the enhancement of Δ**H** within the equatorial electrojet region, and thus the SSC near the dip equator was suggested to have a significant contribution due to ionospheric currents. Akasofu and Chapman[4] have shown that polar geomagnetic storm can greatly enhance the equatorial electrojet current. Rastogi[5] has shown that the changes of H field during SSC at a low-latitude station are due to the imposition of an additional electric field over the equatorial ionosphere causing additional SSC-associated electrojet currents. Onwumechilli *et al.*[6] have shown that during moderately disturbed periods, the polar *Sq* variation ($S_q{}^P$) is enhanced and the electric field associated with it affects the equatorial electrojet by suppressing it. Further, the substorm-associated electric field driving the eastward current in the auroral zone causes an enhancement of the equatorial electrojet. An experimental verification of the coupling between equatorial and auroral electric fields has been recently reported by Kelley *et al.*[7], when the eastward electric field measured at the equatorial station Jicamarca by the Doppler shift of VHF scatter is very well correlated with the westward electric field at auroral station College Alaska measured by balloon-borne detectors.

Woodman[8] has recently described the vertical as well as eastward F-region drifts at Jicamarca. At these regions of the ionosphere, these drift velocities are a direct measurement of the electric field in a direction orthogonal to drift and to the direction of the magnetic field. Thus a technique of continuous monitoring of ionospheric electric fields over

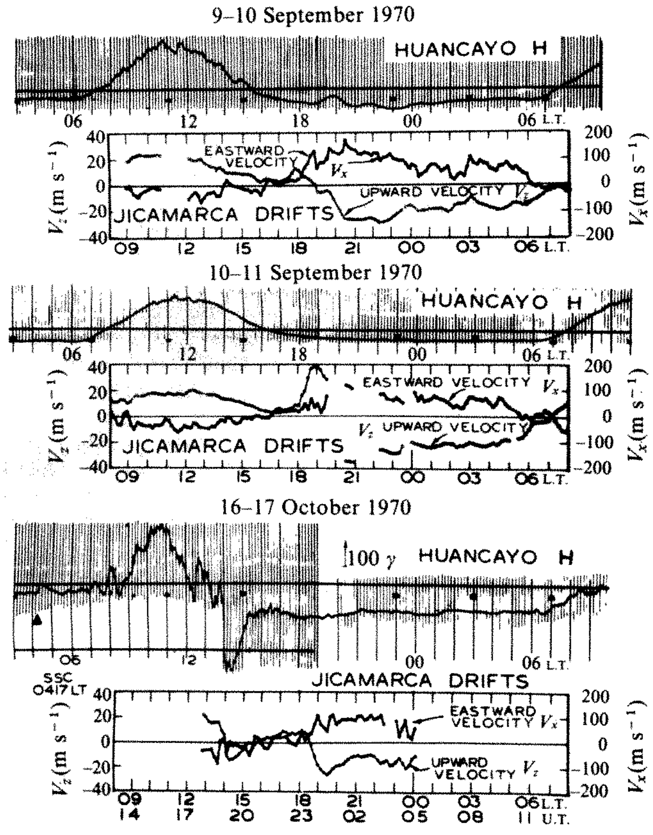

Fig. 1 Comparison of the F region upward and eastward drift velocities at Jicamarca with the magnetograms at Huancayo.

the dip equator has been provided through VHF scatter radar. The data were presented for three days 9–10 September 1970, 10–11 September 1970 and 16–17 October 1970. The first two days, 9–10 and 10–11 September 1970 were geomagnetically very quiet while 16–17 October 1970 was one of the five disturbed days of the month. A sudden commencement type of geomagnetic storm had occurred on 16 October 1970 at 0917 UT (0417 LT). In Fig. 1, these data of upward velocity (V_z) and eastward velocity (V_x) are compared with the corresponding magnetogram of Huancayo.

In Fig. 1, the H field at Huancayo varies very smoothly on 9–10 and 10–11 September 1970. In the evening of 10 September 1970, the vertical drift (or eastward electric field) had a very prominent peak around 1900 LT before the usual evening reversal of its direction. No effect of this sudden increase of the electric field could be seen in the magnetogram because the E region plasma had greatly decreased by 1900 LT and so no significant currents could be produced due to the increase of the electric fielt. On 9–10 September 1970, the evening peak of the vertical drift was absent. The magnetogram for 16–17 October 1970 shows a sudden increase of the H field at 0417 LT due to sudden start of geomagnetic storm. There is a remarkably large and sudden decrease of the H field between 1400 LT and 1530 LT. The vertical drift at Jicamarca shows a large excursion such that the drift was downward between 1400 LT and 1540 LT. Thus, this large decrease of H field near the dip equator during the geomagnetic storm period seems to be due to electric currents in the electrojet region itself due to imposition of a reversed electric field. As described by Woodman[8], the eastward dift shows consistent behaviour from day to day even during a magnetic disturbed day. The variations of the geomagnetic field are further studied in detail in Fig. 2 which depicts the variations of the H field at Huancayo, Fuquene and Huancayo minus Fuquene in American zone and at Kodaikanal, Alibig and Kodaikanal minus Alibig in Indian zone. The corresponding variations of the $\mathbf{D}_{st}(H)$ values, the auroral electrojet (AE) index, interplanetary magnetic field magnitude (**B**) and the latitude (θ) are also shown. The latitude $\theta = -90^\circ$ shows the IMF vector entirely southward while $\theta = +90^\circ$ shows IMF vector entirely northward.

It is seen that the $\mathbf{D}_{st}$ became negative at 1130 UT which signifies the starting of the main phase of the storm. This tallies very well with the variations of **H** at Kodaikanal and at Alibag. The difference fields Kodaikanal minus Alibag is very low during the entire period suggesting that most of the storm effects at Kodaikanal were of magnetospheric origin, and this is consistent with the fact that the storm period was local night for these stations. The onset of MPO does not seem to have affected the Huancayo **H** values which shows a peak before noon. The disturbance field was thus too small to cancel the equatorial electrojet field at Huancayo. At 1400 LT (1900 UT) the H field at Huancayo shows a sharp decrease but no change was noticeable at Fuquene. Thus, this $\Delta\mathbf{H}$ at Huancayo seems to be entirely due to change in the equatorial electrojet current and not due to any changes in the magnetospheric currents. It is remarkable that the Huancayo

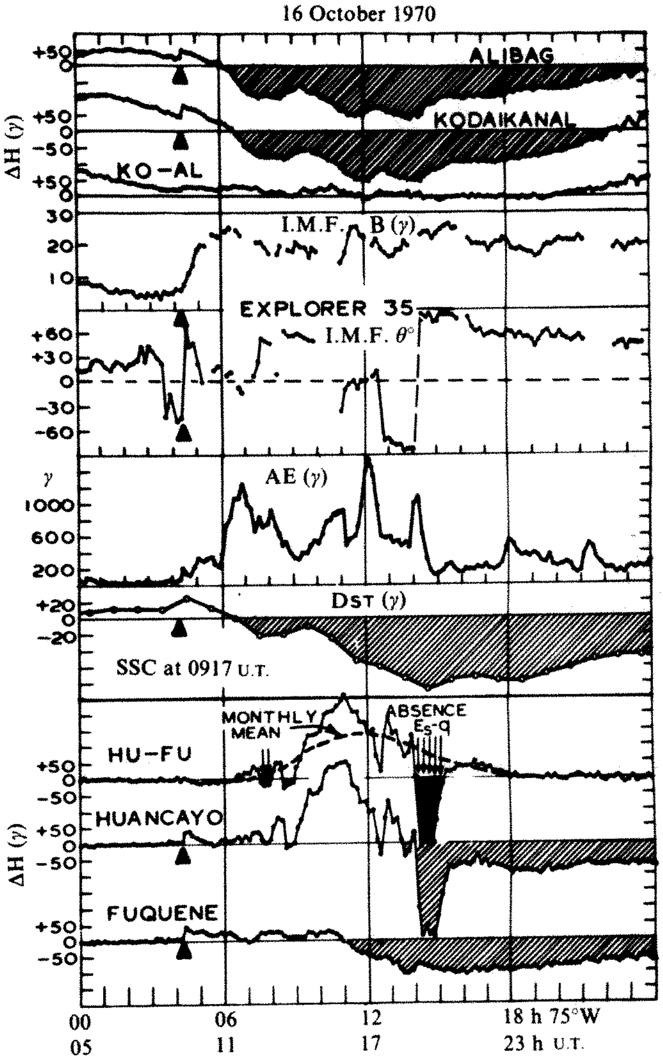

Fig. 2 Comparison of the magnetic field variations at low latitudes during a geomagnetic storm with the auroral index and the interplanetary magnetic field parameters.

ionograms showed absence of the *q* type of E_s layer confirming that the horizontal electric field at the E region over Huancayo between 1400 and 1530 LT was reversed to westward. Examining the AE index, one notices increase of the index around 0700 LT, 1200 LT and 1400 LT. The intense activity of auroral electrojet at 0700 LT did produce some decrease of the **H** at Huancayo between 0800 and 0900 LT. The large increase of AE around 1200 LT decreased the H field around 1230 LT. The most prominent decrease of H field at 1400 LT is associated with the sudden increase of the auroral index at 1400 LT.

Examining the changes of IMF parameters one notices that after the sudden start the magnitude (**B**) had increased from its about usual value of 5 γ to above 20 γ and remained high for the rest of the day. This high value of IMF made the interaction of the field with the solar wind most effective. The direction of the field perpendicular to the ecliptic (θ) shows sudden change at SC and later at 1200 LT. The most remarkable change occurred at 1400 LT when the field flipped from almost the southward to the northward direction. The field remained northward for the rest of the day. Thus the component of the IMF perpendicular to the ecliptic, $\mathbf{B}_z$, component interacting with the solar wind plasma moving practically along the Sun–Earth direction produces *a* $V_x\mathbf{B}_z$ field along the east–west direction. A sudden reversal from southward to northward direction would present a strong westward electric field on the dayside of the Earth. The large increase of the AE index and the reversal of the equatorial electrojet at 1400 LT are ascribed to the sudden flipping of the $\mathbf{B}_z$ component of the interplanetary magnetic field.

Similar effects of the sudden reversals of the $\mathbf{B}_z$ component on the equatorial ionosphere have been previously described by Rastogi and Patel[9]. Similar effects seen during geomagnetic storms show, for the first time, that the observed magnetic field changes near the magnetic equator during geomagnetic storms have significant contribution due to the changes of the electrojet currents in the ionosphere through the modulation of the east–west electric field in the ionosphere. These changes at the equator are suggested to be of the solar wind origin manifested through the changes of the auroral electrojet currents.

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Bimodal distribution of creep event amplitudes on the San Andreas fault, California

EPISODIC fault creep at several instrument sites along the San Andreas and associated faults in central California consists of a few small and large slip events per year generally superimposed on a background of gradual yielding at low rates[1–3]. Most of the events are aseismic, but a few minor displacement steps have occured in association with local earthquakes[1,2]. After removal of earthquake steps, event lists for several sites include significant numbers of small events about an order or magnitude below the typical 1–4-mm amplitude range for large events[1,3]. Recent experimental rock-deformation results demonstrate that under biaxial loading some rocks show episodic slip on pre-cut surfaces[9,10]. It is not yet clear how the laboratory and field observations are related, but the data presented here indicate that episodic fault creep in nature may be more complex than previously realised. In light of the laboratory results, it is more important than ever to consider all the details of the field data concerning fault creep.

The performance of various types of non-wire creepmeters is usually adequate for monitoring large creep events, but these higher friction instruments generally produce incomplete or unreliable results at the level of sensitivity necessary for study of small events[2,12]. Several invar wire creepmeters with low friction and displacement resolution of 0.05 mm or better have, however, been operated nearly continuously within the network since 1972 (ref. 1). At sites characterised by episodic

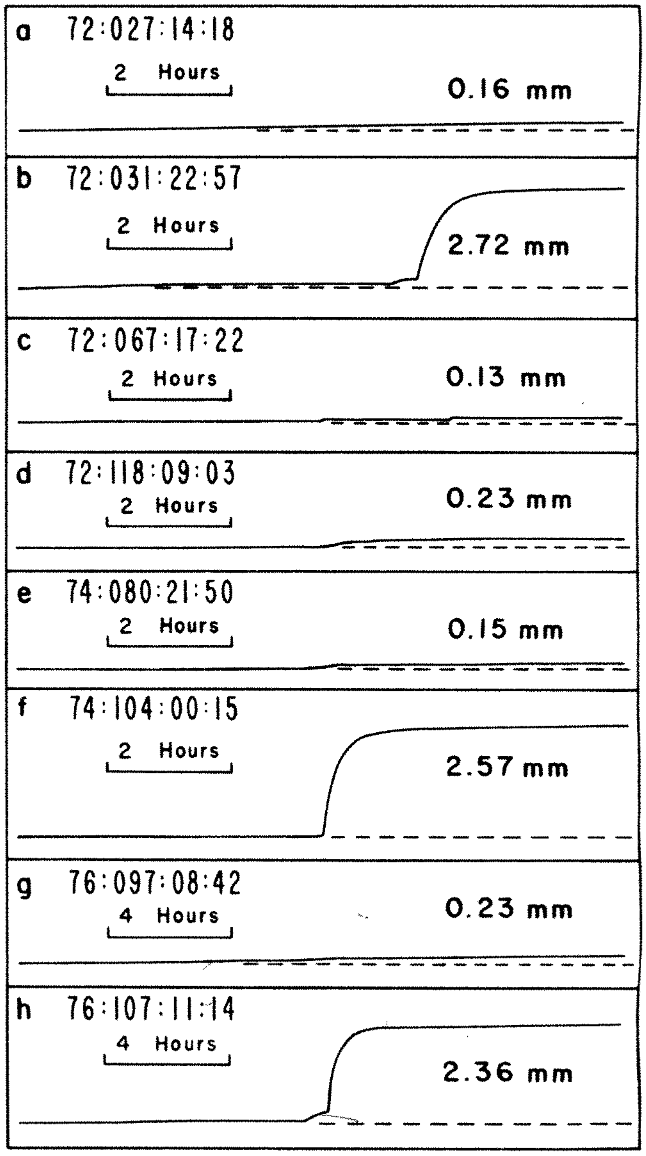


Fig. 1 Tracings from on-site strip-chart records produced at XMR1 creepmeter (36°35.6′N, 121°11.2′W). Onset times for each event are given by yr, Julian d, h and min (GMT). Trace *f* shows a typical large creep event with 80% of the slip occurring in about 1 h. Traces *b* and *h* show similar events except that both are preceded by small precursory movements of about 0.2–0.3 mm. Trace d shows an isolated small event with a rise time similar to that for most large events. Although trace *d* represents the majority of small events, a few with shorter rise times (traces *c* and *e*) and with rise times of several hours (traces *a* and *g*) also occur.

creep, the low-friction wire creepmeters confirm the general pattern of large isolated creep events recorded on the other instruments, but show additional interesting detail concerning the nature of small events.

Records combined from one wire (XMR1)[1,3] and four rod-type creepmeters (MRC, MRB, MRR, MRW)[2,3] clustered along the San Andreas fault trace on Melendy Ranch (36°35′N, 121°11′W) show that most of the large events have rise times of the order of 1 h. These events often propagate at several hundred metres per hour along the entire 1.2-km fault segment covered by the five-station network[1-5,7]. From these and similar observations elsewhere it is clear that large creep events typically involve fault lengths on the order of a kilometre in episodic aseismic slip of a few millimetres amplitude[6,8]. Whatever the actual depth of creep events, it is also clear from consideration of source length alone that considerable volumes of material are involved in the process.

One of the most complete samples of creep event activity is contained in the 1972–76 records from the XMR1 wire creepmeter[1,3]; several examples are shown in Fig. 1. The small events usually have rise times similar to those for large events, but a few small events with shorter rise times (Fig. 1*c*, *e*) and with rise times of several hours (Fig. 1*a*, *g*) are also recorded. The distribution of event amplitudes at XMR1 is distinctly bimodal (Fig. 2). Another interesting feature is that small events at XMR1 preferentially occur during periods of several days just before large events (Fig. 3).

The suprising gap in the distribution of creep event sizes indicates that small events are not necessarily low-amplitude versions of the identical process acting over the same area of fault surface that produces large events. This being the case, there are at least two possible alternatives. The creepmeters may be measuring either the results of the same process operating on two distinctly different scales or the time-displacement signatures of distinctly different failure or propagation processes.

Strong evidence supports the first alternative in that large events usually propagate with a certain degree of regularity across several instrument sites[5,7], whereas small events usually appear at only one creepmeter at a time. Consequently, for small events that are observed, the point at which rupture begins must be very close to the instrument, and the minor slip probably is confined to a relatively small area of the fault surface. The lack of clear evidence for propagation of some of the smallest events over greater distances (≥ 0.5 km), however, could be because their amplitudes are commonly below the resolution of the non-wire creepmeters at sites adjacent to XMR1.

The concentration of small events just before large events indicates that the former are preferentially triggered at stress levels only slightly lower than those necessary for triggering large events. Small events recorded at XMR1 occasionally blend immediately into large events (Fig. 2*b*, *h*), perhaps owing to triggering of slip on a larger fault surface by the initial yielding of a small source area. The time relation reinforces the idea that small and large events result from the same process, with amplitudes determined by the area of the slip surface.

In contrast with this interpretation for the data from central California, results from a strainmeter array in Iran indicate that a small creep event (slip amplitude of about 10 μm) propagated more than 0.8 km along the fault break of the 1968 Dasht-e-Bayaz earthquake, as reported by King and others[11]. The strain results from Iran plus the wider range of rise times for small events than for large events at XMR1 provide some support for the possibility that different failure or propagation processes might be involved.

The explanation for large and small creep events involving a bimodal distribution of source areas implies a heterogeneous distribution of static friction along the fault surfaces that fail in episodic aseismic slip. Large events are perhaps prevented until conditions are favourable for nearly uniform propagation of aseismic slip over the entire large-event source area. The final stages of progress toward this condition probably are marked by a nearly uniform stress level slightly below that necessary for generating large events, but sufficient for initiating minor creep events across small, isolated, relatively weak patches of the fault surface within the source area for the impending large event. The small source areas are viewed as being quasi-permanent in position and dimensions. Slip action would cease after each small event until the general stress reached the threshold value for production of large events. Although many minor-event sources could be widely scattered across the large event area, only those adjacent to or encompassing a creepmeter site would produce detectable offsets. Activity on more distant small-source patches could produce certain small events with extremely long rise times.

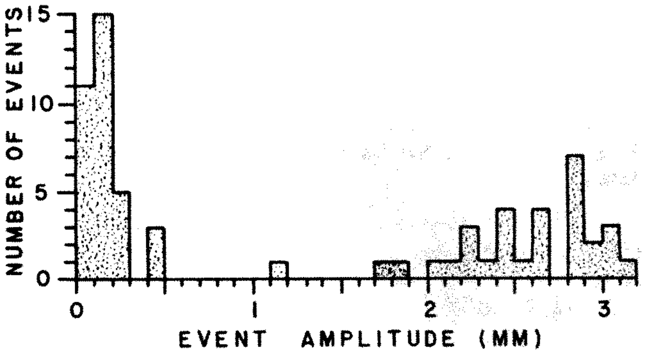
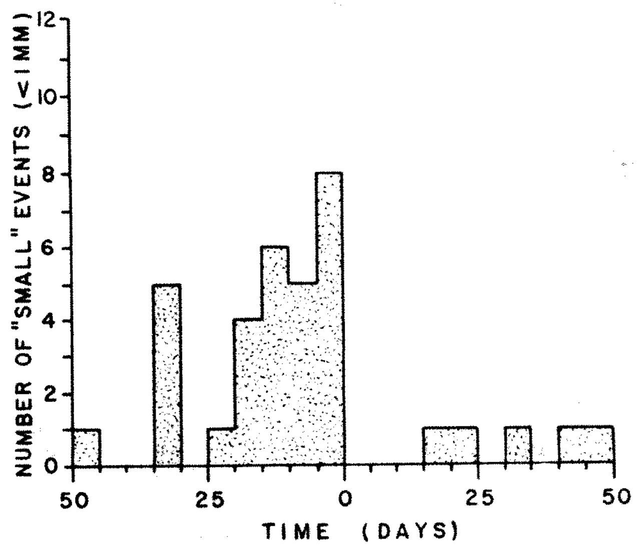


Fig. 2 Histogram of the number of events at wire creepmeter site XMR1 through 16 April 1976, as a function of slip amplitude. Similar bimodal distributions of event amplitudes have been demonstrated for several other sites but are less well documented on non-wire creepmeter records. A decrease in the number of events within the amplitude range 0–0.1 mm compared with the next higher range is probably a reflection of the limits of resolution; a number of small events with amplitudes less than 0.05 mm undoubtedly escape detection.

The expected behaviour of the proposed bimodal source-area system would seem to fit the observed distribution of event amplitudes, the concentration of small events during short periods just before large events, and perhaps the wide range of small-event rise times. The final small event leading to

Fig. 3 Distribution of onset times for small creep events in five-day increments before and after large creep events, from XMR1 records. The zero point on the time scale represents onset times for all large events recorded at the site.



general failure over the large-event source area could occasionally occur near a creepmeter, producing a composite event on the record for that site.

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Loss of boron from shells during weathering and possible implications for the determination of palaeosalinity

The abundance of boron in sediments is frequently taken as an indication of palaeosalinity[1–6]. Because boron is readily absorbed by clay minerals, determinations are generally undertaken on argillaceous sediments, particularly those containing abundant illite, though some analyses are carried out on calcareous sediments. One of the basic assumptions of the boron technique for the determination of palaeosalinity is that there is little or no mobility of boron after deposition, as any mobility might lead to enrichment or depletion of the original boron, and an unreliable value for the palaeosalinity.

A recent study of a Holocene shell sequence from the central Queensland coast indicates a systematic decrease in boron content of the shells with increasing age. This could conceivably be due to a progressive change (that is, decreasing salinity) of the original depositional environment, but other lines of evidence do not support this. It seems more likely that the loss of boron is a consequence of sub-aerial weathering. This suggests that erroneous boron palaeosalinity results may be obtained from calcareous sequences which have been subjected to sub-aerial weathering.

During a study of the sedimentology[7] and geochemistry[8] of sediments at Broad Sound, on the central Queensland coast (Fig. 1), a series of elongate ridges (cheniers) were found, composed predominantly of whole and fragmentary shells. Radiocarbon dating of the shell carbonate revealed that the ridges became progressively older landward, ranging in age from 1,020 ± 70 yr BP to 5,450 ± 85 yr BP (ref. 8). This sequence offered a possible opportunity for quantifying the rate at which geochemical changes take place in calcaleous material under conditions of sub-aerial tropical weathering, or alternatively, for establishing the extent of environmental (temperature, salinity) changes which have taken place in the Broad Sound estuarine system during the last 5,000–6,000 yr. Because the chemistry of shells varies markedly with different phyla and species, the study was restricted to oyster shells, a bulk sample for analysis being prepared from 30–40 small oyster shells of approximately the same diameter, collected from eleven of the cheniers. In addition, modern oyster shells were also collected for analysis from an adjacent intertidal site. All shells were cleaned for 30 minutes in distilled water in an ultrasonic bath before analysis. The results of the boron analyses together with the radiocarbon ages and the proportion of acid-insoluble material are given in Table 1. X-ray diffraction analysis, carried out on all samples showed that the shells are composed of low-magnesian calcite with no significant change in the position of the main calcite peak. But the boron content varies somewhat; it is evident from a plot against radiocarbon age (Fig. 2) that boron decreases with age. Over a period of about 5,000 yr the mean boron content of the shells decreases to 40% of the present day content. Determination of the correlation coefficient indicates that the negative correlation between boron and age has a 99% confidence level.

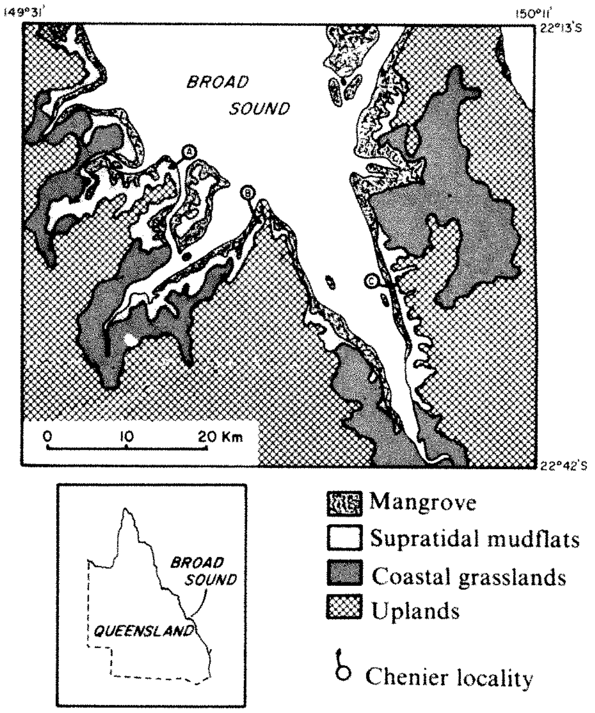

Fig. 1 Map of the Broad Sound area showing chenier locations from which oysters were obtained for this study.

Table 1 Age and composition of oyster shells collected from cheniers, Broad Sound, Queensland

B.M.R. No. 71636	Age (yr BP)	Boron (p.p.m.)	% acid insolubles
205	0	17	0.08
100	1170	15	0.28
141	1640	16	0.04
103	2100	16	0.87
138	2140	15	0.26
202	2480	15	0.13
039	2910	17	0.53
139	2930	14	nd
106	2960	13	0.08
203	3465	12	0.46
107	4060	14	0.15
204	4520	12	nd

nd, Not determined (insufficient sample available).

Radiocarbon ages determined by H. A. Polach, Radiocarbon Laboratory, Australian National University, and R. Gillespie, Radiocarbon Laboratory, Sydney University. Boron was determined spectrophotometrically (using the organic reagent curcumin) by the Analytical Chemsity Section of the Australian Mineral Development Laboratories. Ten replicate analyses indicate a precision of ± 1 p.p.m.

Per cent acid insoluble determined by E. Kiss, Research School of Earth Sciences, Australian National University.

This change in concentration might conceivably be attributable to a progressive increase in salinity at Broad Sound over the past 5,000 yr. Kershaw[9-11] has suggested from palynological evidence that from about 6,000 to 3,000 yr BP the climate of North Queensland was significantly warmer and probably also wetter than the present-day climate. Broad Sound is situated at the confluence of four large estuaries but at the present day there is sporadic freshwater inflow only during the summer months (December to February) and even during this period, measurable dilution in the vicinity of the sampling sites lasts for only a few days. For almost the whole of the year, the water in Broad Sound and its associated estuaries shows complete vertical mixing and has a salinity of about 36 parts per thousand. The increase in rainfall required to have significantly affected the salinity of the Sound would therefore have needed to be of a far greater magnitude than that indicated by Kershaw's work. In addition, comprehensive stratigraphic studies[7], coupled with radiocarbon dating [12], indicate that since sea level stabilised at about 5,000–6,000 yr BP, depositional conditions have remained essentially constant. Therefore, it seems unlikely that the change in boron content can be attributed solely to a progressive increase in salinity at Broad Sound from about 5,000 yr BP to the present.

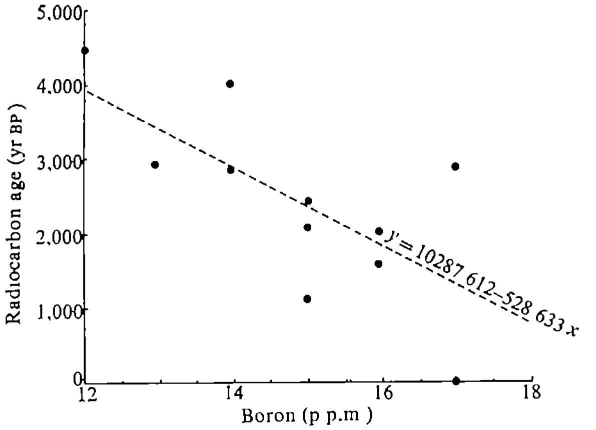

Fig. 2 Plot showing the progressive decrease in the boron content of oyster shells with increasing age.

An alternative explanation is that the decrease in boron content has occurred as a result of weathering. Because of the low concentration of boron in the shells it was not possible to determine the precise location of the boron. Normally, the boron in calcareous sediments is assumed to be in the detrital fraction, a correction factor being applied to determine the palaeosalinity[13]. However, the acid-insoluble fraction (composed entirely of clay-size material) proved to constitute on average no more than 1% by weight of the oyster shells (Table 1). If the mean boron content of non-calcareous clays is taken to be 160 p.p.m. (ref. 6), the acid-insoluble fraction will provide less than 2 p.p.m., or 10–15% of the total boron content of the shells. Also, there is no significant correlation between fraction of acid-insoluble material and the boron content. It therefore, seems most likely that the boron in oyster shells is mainly present within the calcite lattice, probably substituting for carbon. As chemical and X-ray diffraction results indicate that calcium carbonate is not preferentially leached from the shells, it would seem that substituted boron is being preferentially removed from the calcite lattice in oyster shells during the course of sub-aerial weathering. This might take place if the outer portion of the shells was boron rich but there is no reason to suspect that there is any such preferred location. Therefore, for the present it is only possible to point to the loss of boron during weathering without positively identifying the mechanism by which it is removed.

Returning then to the original point regarding the use of boron as an indicator of palaeosalinity. The Broad Sound results indicate that over a period of about 5,000 yr the boron content of oyster shells is considerably reduced. The extent to which these results from oyster shells may be extrapolated to other shells, and in turn to carbonate rocks in general, is uncertain. The results, however, undoubtedly suggest that errors may occur when taking the whole-rock boron content of carbonate rocks as a reflection of the original salinity, if those rocks have been subjected to sub-aerial weathering. It can perhaps be argued that where the boron content of the clay mineral only is considered that the problem of boron mobility is unlikely to arise. The Broad Sound study provides no information on the mobility of boron from clays. However, where the clay is in close proximity to shelly beds, and perhaps calcareous sediments in general, boron mobilised as a result of weathering is likely to be absorbed by the clay minerals. Where there is abundant shelly material this could conceivably result in a marked increase in the boron content of the associated clays, when compared with the original value. Further studies are necessary to fully assess whether the mobility of boron indicated by the Broad Sound study can be extended to calcareous and other sediments, and whether any such mobility limits the application of the boron method for the determination of palaeosalinity.

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Pre-melting near crystal dislocations

IDEALLY, the melting of a crystalline solid should occur at a single temperature. In reality, the process is spread over a small range of temperatures. As the temperature approaches the nominal melting point T_m, the specific heat of, say, bismuth[1,2] acquires a component of the form

$$\Delta C = A/(T_m - T)^2 \qquad (1)$$

where A denotes a constant for a given sample. Usually the premonitory effect is attributed to the presence of impurities that dissolve only in the liquid phase. Indeed, an expression close to equation (1) can be derived on this basis. There is, however, good evidence[3] to indicate that the effect cannot be wholly due to Raoult impurities. In the present letter, we want to show that equation (1) also characterises pre-melting near point defects, while that occurring along edge dislocations is described by

$$\Delta C = B/(T_m - T)^3 \qquad (2)$$

where B denotes another constant.

Slater[4] in his classic paper on the ferroelectric transition of potassium dihydrogen phosphate indicates how a first-order transformation might acquire a temperature broadness at the hands of crystal imperfections. The same mechanism is invoked here to explain pre-melting. According to Slater, the stresses around crystal imperfections alter the effective pressure on the various crystallites and thereby cause them to undergo the transition over a small temperature interval.

To treat pre-melting formally, we can use the continuum model of solids. For mathematical simplicity we take the material to be elastically isotropic. Thus the effective pressure around a point defect varies inversely as the cube of the distance, while that near an edge dislocation goes inversely as the distance. Further, let us assume that the difference in the densities of the two phases is sufficiently small so that any stresses resulting from this difference can be neglected. Since transition curves are practically straight lines, the volume of a liquid domain developing around a point defect should go as $(T_m - T)^{-1}$ while that of a domain growing alongside an edge dislocation should be proportional to $(T_m - T)^{-2}$. Here we identify T_m as the temperature at which a crystallite subject to an effective pressure of 1 atm melts. Finally, using the fact that the change of entropy is proportional to the volume of the domains, we obtain for growth around point defects

$$\Delta C \propto T/(T_m - T)^2 \qquad (3)$$

and for development along dislocations

$$\Delta C \propto T/(T_m - T)^3 \qquad (4)$$

For most solids, the specific heat is anomalous over a temperature interval that is very small compared with the absolute temperature of melting. So relations (3) and (4) are virtually equivalent to equations (1) and (2), respectively.

The present findings lend themselves to a simple experimental test. First the anomalous component of the specific heat of a relatively pure bismuth crystal is determined. Then the crystal is severely deformed and the measurement is repeated. if sufficient dislocations are introduced, then the pseudo-critical exponent of its anomalous specific heat should go from −2 to −3.

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Major genes for copper tolerance in *Mimulus guttatus*

HEAVY metal tolerance in higher plants has proved to be an excellent model system which has provided insight into several aspects of the evolutionary process (see ref. 1 for review). But comparatively little is known about the formal genetics of this character in higher plants, since the grasses which have been the subject of most of the ecological work are not readily amenable to genetic analysis. Antonovics *et al.*[1] argue that the data indicate that tolerance is governed by a polygenic system, though it has been shown that in yeast[2,3] and *Paramecium*[4] that major genes are responsible. The discovery[5] that the yellow monkey flower, *Mimulus guttatus,* has also developed copper tolerant races has enabled this hypothesis to be tested—the genetics of *M. guttatus* are readily investigated since it is easily destaminated and crossed, produces up to 400 seeds at a time, and can complete a generation in less than 100 d. The data presented here suggest that there are only two genes responsible for most of the tolerance observed in this plant.

A single plant (C10), typical of one of the most tolerant Californian populations, Copperopolis 4C, was crossed with a plant from the non-tolerant population at Cerig-y-drudion (for details of the populations see ref. 5). Forty F1 plants were tested for copper tolerance, and the most and least tolerant plants were then backcrossed to a Cerig plant. Twelve individuals of each of the F1 and the two backcross progenies were then grown in each of two fully randomised blocks. C10 was cloned, and 15 ramets of this plant and 15 Cerig individuals were also included in the randomisation. Because of the accidental loss of many of the plants from one block, a third block was later raised with 20 of each family. The data from all three blocks was pooled. Thus each family is represented by a maximum of 44 individuals. But, due to the death of some plants, and the fact that many individuals could not be tested at both levels of copper used in this experiment, the number of plants tested at any level is less than this.

The most immediate effect of heavy metals on non-tolerant plants is the inhibition of root growth[6]. This phenomenon provides the basis for the method of testing tolerance. Usually[7,8] an index is produced as the ratio of growth in a test solution to growth in a control solution, but in this instance, since we are concerned with the progeny of a single plant, the mean root growth of the

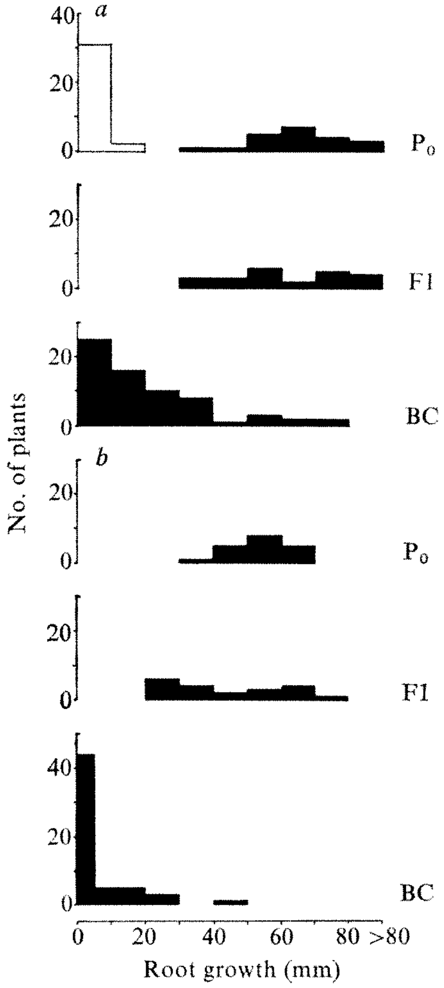

Fig. 1 Histograms of rootgrowth of P_0 (C10 filled, Cerig open), F1 and combined backcross (BC) progenies at *a*, 0.125 and *b*, 0.5 p.p.m. copper. Cerig plants were only tested at 0.125 p.p.m. since they all produce roots shorter than 2 mm at the higher level. The root growth of each plant is the mean of two cuttings grown for a week in a solution of 0.5% (w/v) $Ca(NO_3)_2$ containing the requisite level of copper as $CuSO_4$. Solutions were changed on the third and fifth days. Testing was performed at 23±1 °C with constant illumination. Note that not all plants could be tested at both levels. Full experimental details are given in ref. 9.

longest roots of two cuttings in a solution containing either 0.125 or 0.5 p.p.m. copper is used as the measure of tolerance. This method has the advantage that non-tolerants are more easily distinguished since at 0.125 p.p.m. they form short stumpy roots, while at 0.5 p.p.m. any roots which are produced die almost immediately.

Figure 1 gives the distribution of tolerance of the two parents, their F1 and the two backcross progenies, which have been pooled since they did not differ significantly (see Table 1*b*). At 0.125 p.p.m. it is apparent that the backcrosses contain many non-tolerant plants, but further conclusions at this level are limited by the large error inherent in measuring tolerance by this method. This error is revealed in Table 1, which gives the analysis of variance for each family at 0.125 p.p.m. More than 50% of the total variance of each of C10, Cerig and the F1 is due to measurement error (within plant variance). This variation is due to the natural variability of root growth: the principal source seems to be variation in the day of initiation (unpublished), though other factors are probably important (for example see ref. 10). Considerable genetic segregation in the backcrosses is indicated by the far larger proportion of its variance due to differences between individuals. Table 1 also shows that the C10, Cerig and F1 plants do not seem to be uniform at this level. The C10 ramets are genetically identical, and this variation is inexplicable in this instance. It is not found at other levels of copper in this clone, nor in two other clones tested. Significant differences within the Cerig population and the F1 family indicate that not all the individuals in these families are identical. In the case of the F1 this may indicate that C10 was heterozygous for some loci, but the lack of significant difference between the two backcross families indicates that this segregation is minor compared with the segregation in the backcrosses of loci homozygous in C10.

But at 0.5 p.p.m. Cerig plants are unable to form roots. Any that start to grow turn dark brown and die almost immediately. Any plant that shows any significant root growth must therefore be tolerant to a degree. The large error involved in tolerance testing is no longer so important because at this level we can make a qualitative distinction between tolerants (those plants which form roots) and non-tolerants (those that do not). All F1 plants are tolerant, but the backcrosses are segregating a large number of plants which effectively fail to root.

On this basis, the number of tolerant plants is 14, while there are 44 plants which are non-tolerant at this level. This ratio differs significantly from 1 : 1 ($\chi^2 = 15.5$), but not from 1 : 3 ($\chi^2 = 0.02$). A one gene model anticipates two equally frequent classes of progeny in the backcross, while a two locus model predicts three (AaBb; Aabb and aaBb; and aabb) in a 1 : 2 : 1 ratio, or 1 : 3 if the latter two classes cannot be distinguished. The data thus concord with a two-locus model, but not with a single gene hypothesis. The data at 0.125 p.p.m. can also be explained on a two locus model. Figure 2 shows the relationship between the tolerances at high and low levels of copper of plants tested at both levels. It is evident that, despite the high variation in testing, the correlation between tolerance at 0.125 p.p.m. and 0.5 p.p.m. is high. The plants which prove to be tolerant at 0.5 p.p.m. are mostly the plants with the highest tolerances at 0.125 p.p.m. But, there are still many plants which show greater root growth than Cerig plants at 0.125 p.p.m. but which show none at 0.5. On a two-gene hypothesis, in which only plants with both genes are tolerant, there is the problem of what the phenotype of a plant with only one gene is. These data would suggest that it could be partially tolerant at low levels of copper, but non-tolerant at higher levels.

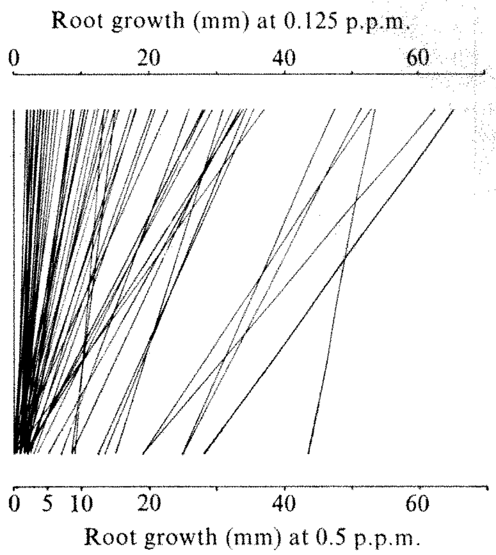

Fig. 2 The correlation between rootgrowth at 0.125 and 0.5 p.p.m. of the combined backcross progenies. All plants tested at both levels are included.

Models involving more than two genes of roughly equal effect would need to be complicated in order to fit these data. With three genes there are four classes with 0, 1, 2 and 3 tolerance genes respectively, and these are expected in a 1 : 3 : 3 : 1 ratio: it is difficult to see how the data can be explained on such a segregation. It is also apparent that at 0.125 p.p.m. there are many plants which are as non-tolerant as Cerig plants: the segregation of plants with no tolerance genes at all becomes progressively rarer as the number of genes postulated is increased.

The data presented here indicate that there are two major

Table 1 Analysis of variance at 0.125 p.p.m. of *a*, C10, Cerig and the F1, and *b*, the backcross families

Source	d.f.	C10 MS	*F*	d.f.	Cerig MS	*F*	d.f.	F1 MS	*F*
a									
Between plants	18	420.85	2.79*	32	19.51	2.06*	22	731.41	2.62*
Within plants	19	150.68		33	9.45		23	279.50	
$\frac{\text{Error MS}}{\text{Total MS}}$ (%)		53%			65%			55%	
b		Backcross families							
Between families	1	22.46	0.03NS						
Between plants	64	748.21	7.70†						
Within plants	66	97.11							
$\frac{\text{Error MS}}{\text{Total MS}}$ (%)		23%							

In the case of C10, the different plants are all ramets. In all the other families, the different plants are all different individuals. d.f., Degrees of freedom; MS, mean square; *F*, variance ratio.
*Significant at the 5% level.
†Significant at the 0.1% level.

genes involved in the production of copper tolerance in the Copperopolis population of this species. This conclusion is being tested by further breeding of the backcross progeny. However, these data do not rule out the possibility of there being other loci of lesser effect in addition. That this is likely is shown by the fact that crosses within the Copperopolis population reveal genetic variation[9]. All the Copperopolis plants tested were tolerant at 1.0 p.p.m., however, indicating that they were all homozygous for the two major genes.

This result has important physiological implications. Early work[11–13] on the physiology of tolerance suggested that zinc was bound up in the cell walls of zinc tolerant plants, thus preventing the metal from inhibiting cellular enzyme systems. Wainwright and Woolhouse[14], however, argue that this may not be the only mechanism, and showed that individual enzyme systems may show tolerance. These results were not repeated in studies on different systems[15,16]. If enzymatic adaptation were a major component of tolerance one would expect that there would be many genes involved in producing it, because heavy metals are general protein inhibitors. Unless there is one enzyme system which is much more susceptible to copper than others, the substitution of a single non-tolerant enzyme by a tolerant form is unlikely to increase the tolerance of the whole phenotype by a large amount. A compound that was able to bind heavy metals and reduce their effective cellular concentration could make a significant contribution to the overall tolerance of an individual, however. It is possible that the production of such a compound could be mediated by a few genes. Thus the finding that there seem to be only two genes in *M. guttatus* producing most of the tolerance observed in the highly tolerant Copperopolis population tends to implicate a primary physiological mechanism involving the production of a protein able to bind copper and render it unavailable to the cell, rather than a system of general enzymatic adaptation.

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Association of alleles at esterase loci in wild barley *Hordeum spontaneum* L.

THE aim of the conservation of plant genetic resources is to collect and preserve representative samples of genetic variation from the primitive 'landraces' and the related wild species of crop plants on which man depends for use in future breeding programmes[1,2]. Counter proposals[3] that breeders obtain specific alleles by mutagenesis at will are defensible when one is considering single loci. But groups of alleles at many loci which have jointly been subjected to natural selection, are unlikely to be assembled by mutation breeding. We report here the widespread occurrence of co-adapted combinations of alleles at the four esterase loci in populations of wild barley (*Hordeum spontaneum*) in Israel, thus reinforcing the case for genetic conservation.

Cultivated barley (*Hordeum vulgare*) is polymorphic for four esterase loci. Three of the loci, denoted *Est A*, *Est B* and *Est C* are tightly linked ($B \leftarrow 0.0023 \rightarrow A \leftarrow 0.0048 \rightarrow C$) and a fourth, *Est D* recombines independently[4]. In composite cross V, Allard and co-workers have described changes in gene frequency at these loci[5], and have shown that only a few specific combinations of alleles were favoured[6]. Similarly in composite cross II, two balanced arrays account for 33% of the gamete pool[7] after 26 generations. These esterase loci are also highly polymorphic among the accessions in the US Department of Agriculture barley collection[8] of the ancestral species (*Hordeum spontaneum*) (ref. 9). Here we report that natural populations of wild barley in Israel also exhibit only a restricted number of complementary arrays of alleles at the esterase loci.

Single seedlings from about 50 panicles sampled from each of 11 populations were subjected to starch gel electrophoresis. The populations form transects running east to west (numbered 1–8 in Table 1), and north to south (9–11) across Israel. All loci were scored in a leaf sample, and *Est A* and *Est C* were scored in a separate root sample. *Est D* was scored in only three populations. The alleles were assigned to mobility classes with respect to the commercial cultivars Atlas (SMFM), and Clipper (MMFS). (SMFM and MMFS refer to slow (S), medium (M) and fast (F) 'mobility classes' of esterases specified by loci *A*, *B*, *C* and *D* respectively when subjected to starch gel electrophoresis.) Table 1 lists the frequencies (f_{ijk}) of the two most common gametic arrays in each population. Thus, for example the 60% of the gametes in the Damun population were FSFM, 16% were MSMF and the remainder carried less frequent arrays. The observed number of alleles at each locus and the information at locus *X*

$$(I_X\ (= -\Sigma_j p_j \log_2 p_j))$$

(ref. 10) are listed. Most populations are appreciably polymorphic. However, the observed average heterozygosity was less than 0.01, because the outcrossing rate (t) was low ($0 < t < 0.02$).

Table 1 also lists estimates of two and three locus measures of allelic association at the specified esterase loci. These logarithmic measures are computed as $I_{XY} = \Sigma f_{ij} \log_2(f_{ij}/p_i p_j)$ ($f_{ij} > 0$) for two loci, and $I_{XYZ} = \Sigma f_{ijk} \log_2(f_{ijk}/p_i p_j p_k)$, ($f_{ijk} > 0$) for three loci[11,12]. There is a very high level of complementary association. For example, at Damun $I_{AC} \simeq \min(I_A, I_C)$ which is the theoretical maximum association in this case. Also $I_{ACD} \simeq I_{AC} + I_{CD}$ which is its theoretical maximum given the two locus values. The quantity ($1.38\ NI$) where N is the number of independent gametes in the sample ($N \simeq 50$), is distributed asymptotically as χ^2 with appropriate degrees of freedom. The values of I can be tested for departure from zero or lack of association. Virtually all tests indicated statistically significant association. Intense association between alleles over loci have been reported in natural Californian populations of wild oats[13]. There is also significant association between esterase loci in animals (salamanders[14] and *Drosophila montana*[15]).

The data from wild barley presented here highlight two important difficulties of interpretation which cannot be resolved unequivocally. First, there is disagreement concerning the extent to which allozyme polymorphisms are maintained by natural selection[16]. Furthermore, it is recognised in addressing this question, that the loci cannot be considered separately from the chromosomal segments to which they belong. Second, when there is total self-fertilisation, selection is not necessary to preserve associations of alleles between loci, since this mating system is sufficient to retain any associations generated sporadically by mutation or random drift and partial isolation[17].

Table 1 Esterase polymorphism in eleven population of *Hordeum spontaneum*

	Population		Most common gametes and their frequency (f_{ijk})		Number of alleles and information (I) at esterase loci				Two and three locus measures of association for specified combinations of esterase loci			
					A	*B*	*C*	*D*				
1	Akhziv	Coastal	MFS	0.42	1	4	3		*BC*	0.97**		
			MMF	0.21	0	1.70	1.36					
2	Maalot	Montane	MFS	0.88	1	4	3		*BC*	0.57**		
			MVF	0.06	0	0.7	0.57					
3	Mt Meron	Montane	MFS	1.00	1	1	1					
					0	0	0					
4	Zefat	Montane	MFS	0.69	1	4	3		*BC*	0.84**		
			MSM	0.20	0	1.16	1.00					
5	Tel Hai	Foothills	MVS	0.59	1	3	2		*BC*	0.98**		
			MSM	0.29	0	1.33	0.98					
6	Rosh Pinna	Foothills	MPM	0.66	2	4	2		*AB*	0.66**	*BC*	0.66**
			FIM	0.17	0.66	1.38	0.66		*AC*	0.05	*ABC*	1.32**
7	Gadot	Rift Valley	MIM	0.27	4	5	3		*AB*	0.39**	*BC*	1.18**
			MVS	0.23	0.77	2.30	1.51		*AC*	0.05	*ABC*	1.58**
8	Shifon	Montane	MMS	0.85	2	2	1		*AB*	0.01		
			MVS	0.10	0.19	0.48	0					
9	Damun	Montane	FSFM	0.60	2	1	3	3	*AC*	0.90**	*CD*	0.45*
			MSMF	0.16	0.90	0	1.21	1.12	*AD*	0.25**	*ACD*	1.35**
10	Bar-Giora	Montane	MFMM	0.44	2	3	2	1	*AB*	0.48**	*BC*	0.99**
			MMSM	0.46	0.66	1.38	0.99	0	*AC*	0.10*	*ABC*	1.48*
11	Yeruham	Steppe	MMVF	0.86	1	1	3	3	*CD*	0.26**		
			MMSM	0.04	0	0	0.47	0.82				

*Association significant at the 5% level.
**Association significant at the 1% level.

However, provided there is some outcrossing and recombination, strong associations cannot persist in equilibrium populations if the chromosomal segments are neutral to selection[18,19]. We conclude that the occurrence of allelic associations for marker loci in natural populations of the wild relative of a crop plant is an observation crucial to the rationale of genetic conservation.

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Prediction of seed longevity at sub-zero temperatures and genetic resources conservation

THE International Board for Plant Genetic Resources (IBPGR) is encouraging the development of an international network of seed banks for the conservation of the genetic resources of the major crop species of the world and their wild relatives[1]. Most of the species in priority lists show 'orthodox' seed characteristics[2], that is their longevity is prolonged in a defined manner by a reduction in moisture content (MC) (at least down to 5% MC, fresh weight basis) and temperature[3]. From a consideration of biological factors and the design and cost of seed storage facilities, IBPGR recommends that orthodox seeds are stored at −18 °C or less and at $5\pm 1\%$ MC[2]. It is convenient to aim at −20 °C as the nominal temperature. Because of the accumulation of mutations associated with loss of viability[4,5], IBPGR recommends regeneration of stocks (that is growing plants to provide new seeds from the old) when viability has fallen by 5% (ref. 2). To plan ancillary facilities and procedures, it is necessary to estimate regeneration intervals (time taken for viability to fall by 5%) for the major crop species. Such estimates pose three problems: there are genotypically controlled variations in longevity within a species; seed 'quality', determined by conditions before harvest and during processing, can affect subsequent longevity; and loss of viability at −20 °C is extremely slow, and so far it has been possible to predict regeneration interval only by questionable extrapolation from more favourable storage conditions[4,6]. We have examined these problems in barley (*Hordeum distichum*, L.) and have developed an approach which should make possible more accurate predictions from experiments lasting only 2 yr—even though fresh seed of most species shows no detectable loss of viability during this time.

Loss of viability in orthodox seeds conforms closely to three basic viability equations applicable over a wide range of conditions in all seven species that have been examined in detail (Table 1) as follows[3,6]. The frequency of individual deaths in time in a population stored in constant conditions is described by the normal distribution

$$y = \{1/\sigma\sqrt{(2\pi)}\}\exp\{-(p-\bar{p})^2/2\sigma^2\} \quad (1)$$

where y is the relative frequency of deaths occurring at time p, $\bar{p}$ is the mean viability period, and σ is the standard deviation of the distribution of deaths in time. Consequently survival curves (percentage viability plotted against time) are cumulative normal distributions of negative slope, which become straight lines if transformed to probit (as in Figs 1 and 2). The spread of the distribution is directly proportional to the mean viability period, that is

$$\sigma = K_\sigma\bar{p} \quad (2)$$

where K_σ is a constant. And the relationship between moisture content, temperature and mean viability period is described by the equation

$$\log\bar{p} = K_v - C_1 m - C_2 t \quad (3)$$

where m is moisture content (% fresh weight basis), t is temperature (°C), and K_v, C_1 and C_2 are constants. Thus once the four viability constants (K_v, K_σ, C_1 and C_2) have been determined it is possible to define percentage viability after any period of storage in any combination of conditions.

We have examined five cultivars of barley (Vada, Berac, Julia, Golden Promise and Proctor) in various storage treatments and have found that the C constants have identical values ($C_1 = 0.098$ and $C_2 = 0.050$). Genotypic differences in longevity are attributable to differences in the value of the K constants: K_σ varies only slightly (mean value 0.214), but there are quite large differences in K_v, varying from 4.69 (when $\bar{p}$ is expressed in days) in Julia, to 5.56 in Proctor. Since K_v is related to log $\bar{p}$, this difference results in a sevenfold variation in longevity between the cultivars in identical storage conditions and, because the values of the C constants are identical, it follows from equation (3) that this relative difference is maintained in all storage conditions.

In experiments using Proctor we have investigated treatments on the seed crop which could affect subsequent viability, including nitrogen nutrition (15 or 70 kg ha^{-1} N), shading (intercepting 60% radiation for a month before harvest), degree of unripeness, and severity of threshing. Only shading and marked unripeness significantly affected longevity. As with genotypic effects, only the K constants were affected (K_σ only slightly). K_v was reduced from 5.56 to 5.54 by shading and to 4.70 by harvesting unripe seed. Thus only unripeness had a major effect, but this was as much as could be caused by genotypic effects. Now that it is known that both genotypic and prestorage environmental factors only have any practical effect on K_v, further estimates of possible variation in longevity within a species are greatly simplified.

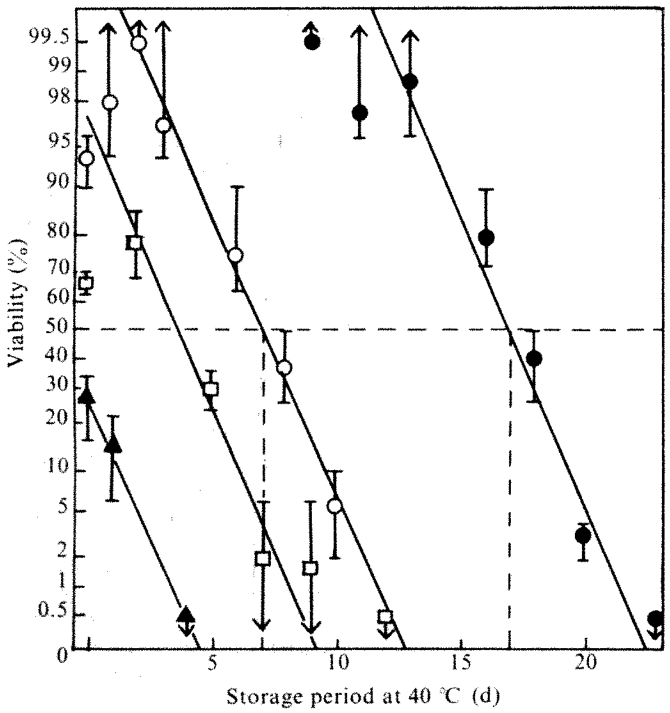

Fig. 1 Survival curves of barley seed (*H. distichum* cultivar Proctor) at 15.4% moisture content in hermetic storage at 40 °C after 0 (●), 3 (○), 4 (□), and 5 (▲) d previous storage at 50 °C. Percentage viability (mean of five replicates of 50 seeds each) is plotted on a probability scale. The vertical bars represent the extreme values of the replicates. Since 100% and 0% cannot be shown on a probability scale, such values are represented by arrows pointing upwards at 99.5% or downwards at 0.5%. Germination tests to determine viability were carried out at 7 d at 3 °C (to remove dormancy) followed by 14 d at 20 °C. Extension of the radicle by > 2 mm was taken to indicate a viable seed. Note that 3 d of preliminary storage at 50 °C had no significant effect on initial percentage viability, but decreased the mean viability period from 17 to 7 d.

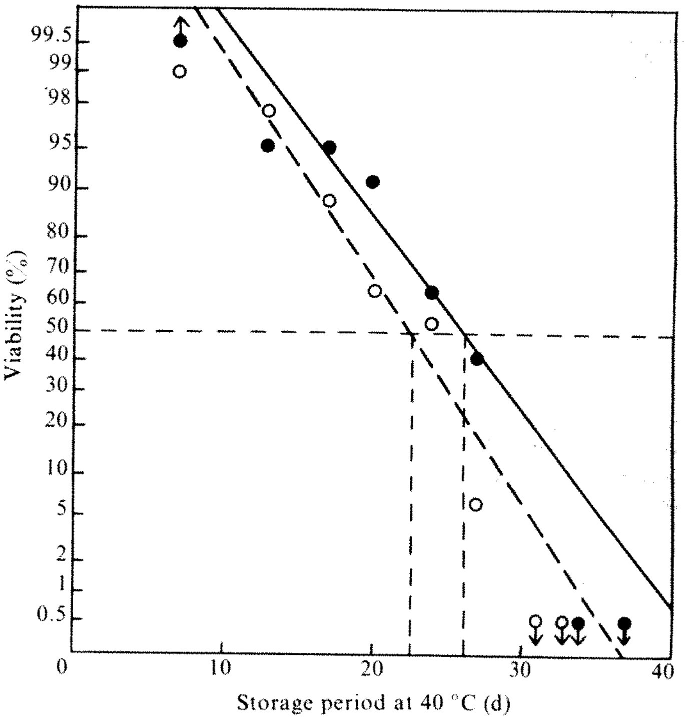

Fig. 2 The effect of 750 d of hermetic storage at −20 °C and 15.4% moisture content on the viability of barley seeds (*H. distichum* cultivar Proctor). Seed at 15.4% moisture content was hermetically stored at 40 °C. Paired samples were withdrawn at intervals and viability immediately determined on one sample (●). The second sample in each case was stored for a further 750 d at −20 °C after which viability was again determined (○). Each point represents the mean of four replicates of 50 seeds. Further explanation as for Fig. 1.

To develop a technique for estimating the very slow rates of deterioration which occur at sub-zero temperatures, it first had to be confirmed, using alternating temperature regimes, that change in temperature *per se* had no effect on longevity. It was then established that any preliminary seed deterioration did not affect the subsequent slope of the survival curve, but only the intercept: slope is determined solely by contemporary conditions (Fig. 1). Figure 2 shows that although 750 d of storage at −20 °C had no significant effect on initial viability, as would be expected from the shape of the survival curve, there is a maximum affect on percentage germination on samples approaching 50% viability. From the $\bar{p}$ values shown by the vertical broken lines, it can be calculated that 750 d of

Table 1 Provisional estimates of probable regeneration intervals for seeds stored at −20 °C and 5% moisture content

Species	Cultivar	Viability constants* K_σ	K_v	C_1	C_2	Probable regeneration interval (yr)†
Barley (*Hordeum distichum*)	Proctor, Golden Promise, Julia (mean values)	0.214	4.800	0.098	0.050	70
Rice (*Oryza sativa*)	Norin	0.200	5.680	0.150	0.047	300
Wheat (*Triticum aestivum*)	Atle	0.350	5.067	0.108	0.050	78
Broad bean (*Vicia faba*)	Claudia superaquadulce	0.379	5.766	0.139	0.056	270
Pea (*Pisum sativum*)	Meteor	0.384	6.432	0.158	0.065	1,090
Onion (*Allium cepa*)	White Portugal	0.486	4.906	0.107	0.055	28
Lettuce (*Lactuca sativa*)	Grand rapids	0.110	3.805	0.079	0.047	11

*Sources of values for viability constants: barley[7]; rice, calculated from figures supplied by Dr H. Ito[8]; wheat[9]; broad bean and pea[10]; onion[7]; lettuce, Dr Ann Minchin (unpublished results).

†Probable regeneration interval is calculated as predicted time for viability to fall to 95% of initial value (p_{95} period). Because 95% of the area under a normal curve (that is 95% of seed deaths) occurs when storage period $>\bar{p}-1.645\sigma$, it follows from equation (2) that $p_{95}=\bar{p}-1.645K_\sigma\bar{p}$. The value of $\bar{p}$ was solved by inserting the appropriate viability constants in equation (3) at 5% MC and −6 °C. The value −6 °C rather than −20 °C was chosen on the basis of the preliminary work on barley which indicated that equation (3) underestimates rate of loss of seed viability at sub-zero temperatures.

storage at −20 °C $\hat{=}$ 3.8 d of storage at 40 °C, that is, 1 d at 40 °C $\hat{=}$ 197 d at −20 °C. If C_2 has the same value at −20 °C, the predicted temperature, t_p, at which this result should have been obtained is given by $t_p = 40-(\log 197/C_2)$, that is −6.3 °C. Thus it seems that the rate of deterioration at −20 °C was greater than would have been predicted by extrapolation from higher temperatures.

These results have suggested an improved technique which we believe should be applied to the major crop species to estimate regeneration interval. It depends on the strong evidence that the value of K_σ remains the same in all storage conditions[3,7]. If a sample were reduced to about 50% viability by rapid ageing (for example, storage at 40 °C at about 15% MC), it could then be stored at −20 °C for, say, 2 yr when significant losses of viability would be expected. Sequential germination test over this period would determine the slope of the survival curve from which σ, $\bar{p}$ and K_σ could be calculated (equation (2)). Similar treatments at several other moisture contents and sub-zero temperatures would provide additional values of $\bar{p}$, making possible simultaneous solution of equation (3) and providing the values of all four viability constants determined in conditions approximating those recommended, and avoiding the need to extrapolate from higher temperatures.

Further work is needed, but will take a few years to complete. Meanwhile practical decisions have to be made, and it is suggested that at present best estimates of regeneration interval are obtained by applying the three viability equations and the four viability constants determined at higher temperature to predict the time taken for viability to fall by 5% at −6 °C (because extrapolation to −20 °C seems to underestimate the rate of deterioration at that temperature). Although such estimates (Table 1) may be far from perfect, they suggest that in the conditions recommended by IBPGR, seeds of many species are stored without regeneration for more than a century; but some species may need to be regenerated in less than 20 yr. Because longevity is affected by both genotype and pre-storage environment, it is essential that stocks are monitored for loss of viability at intervals which relate to expected longevity. It is not sufficient, however, to be guided entirely by the expected time for viability to fall by 5% (p_{95} period). The seed-to-seed variation in longevity (described by K_σ) which determines the slope of the survival curve is also important, because a species with a low K_σ value will give relatively little warning of an impending fall in viability (for example, lettuce in Table 1), and such species should be monitored more frequently.

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Night flight towards a sex pheromone source by male *Spodoptera littoralis* (Boisd.) (Lepidoptera, Noctuidae)

Pheromones have been used in population monitoring traps[1] and in control by 'confusion'[2], and a good understanding of how the male finds the female is of great practical importance in the development of these techniques. The problem of insect orientation towards distant odour sources is also intrinsically interesting and much work has been done with wind tunnels to study the flight of moths in attractant plumes (the most recent of many reports is ref. 3). These studies have shown that moths fly upwind in a series of horizontal zig-zags which decrease in amplitude as the source is approached. Field observations support these general findings[4], but they are not as detailed as the results of laboratory studies and the techniques are not suitable for night flying species, many of which have considerable economic importance. We describe here techniques for observing and recording the details of the behaviour of night flying insects in attractant plumes, and also some new findings which suggest that flight towards a sex pheromone source in one species consists of several distinct stages with deviations from a straight upwind path taking place largely in the vertical plane.

The work was carried out in Crete during August and September 1975 and 1976 as part of experiments on the use of sex pheromones to control *Spodoptera littoralis* (Boisd.), a noctuid moth, the larvae of which damage many crops, including lucerne and cotton. For flight trials the synthetic sex attractant pheromone *cis*-9, *trans*-11-tetradecadien-1-yl acetate[5] was used, dispensed from a cylindrical polythene vial 3 cm long and 1.5 cm in circular cross section diameter, giving a release rate of order 5 ng min^{-1}. This was suspended 1 m above the ground in lucerne 0.2–0.3 m high. Even in bright moonlight *S. littoralis* males cannot easily be seen beyond about 2 m and to see moths at greater distances a night-viewing device (NVD) was used. This consists of a cascaded set of photomultiplier tubes, giving a light magnification of order ×40,000 (ref. 6) and optics which produce an image magnified ×2.5. In field conditions a grey object of 5 cm^2 can normally be seen at 25 m in bright starlight. The instrument has a very large object lens, however, and therefore a large effective aperture; at the desired ranges, of order 20 m, the depth of field is inadequate for small moving targets. Also the image at low light levels is coarse grained and scintillates strongly. Both of these limitations have been overcome by using supplementary infrared illumination which greatly improves image brightness and definition in the NVD and facilitates the use of aperture disks effectively to 'stop down' the object lens, thereby increasing depth of field. With a 4-mm aperture, the depth of field at 25 m is about 10 m. Two 55-W car spotlights were placed 16 m apart on the windline through the attractant source and shining towards each other and the source, with the upwind lamp some 6 m from the attractant. The beams were filtered by infrared gelatine filters (Wratten 87) which nominally pass light in the wavelength range 750–1,000 nm only, well beyond the sensitivity found in the eyes of most insects except at very high intensity[7].

Flight behaviour was observed both from a position 10–20 m to the side of the expected flight path and from upwind of the source looking along the path. Portions of the flight path were recorded in side view by two methods. A 16-mm cine camera recorded the NVD image, and infrared-sensitive film was used in a 35-mm still camera with a sector shutter giving a series of images of the moth's flight on one frame. Figure 1 shows the last few metres of a flight as an example of the latter method, and Fig. 2 shows a reconstruction of a flight path made by superimposing drawings of the moth's position taken from a series of consecutive frames of cine film. From the measured and scaled time interval, the actual speed of the insect along its flight path relative to the ground (the 'speed of flight') and its horizontal component, the ground speed, can be calculated. Both techniques make it possible to see the heading of the moths and on the 35-mm frames the body angle of attack can also be seen.

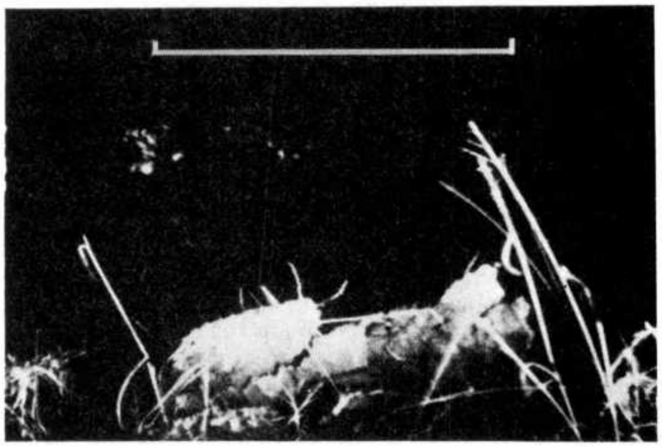


Fig. 1 Multi-exposure infrared photograph showing 'undulating' and 'hovering' flight. Scale shown is approximately 1 m. Inverval between successive frames was approximately 1/30 s. Wind was from the left.

Male moths showed a surprisingly consistent flight pattern and in favourable conditions (a steady wind greater than 0.25 m s^{-1}) distinct successive stages of flight were found, performed by some 90% of about 1,000 moths identified as *Spodoptera littoralis* on the strict criterion of their halting their forward progress on the pheromone capsule or in the air close to it. (Some insects of about the size of *S. littoralis* flew straight upwind past the capsule without any apparent recognition of it; it is possible that some of these may have been *S. littoralis* males, but they were discounted.)

Figure 3 shows the general flight pattern. In steady wind the moths were first seen in straight and level flight, making rapid progress directly upwind with an estimated ground speed of 3 m s^{-1} and at an altitude of between 0.5 m and 2.0 m; this portion of the flight was outside the field of view of the cameras. At a distance from the source usually within the range 2–4 m, and apparently fixed for a given wind speed, the moths abruptly reduced both ground speed and 'speed of flight', performing subsequently a slower, more undulating flight of about 0.5 m s^{-1}. This is shown in Fig. 1 up to about 0.5 m from the source and in Fig. 2 up to about 0.2 m. Although the flight path contained some deviations in the horizontal plane, well defined zigzagging took place only as undulation in the vertical plane.

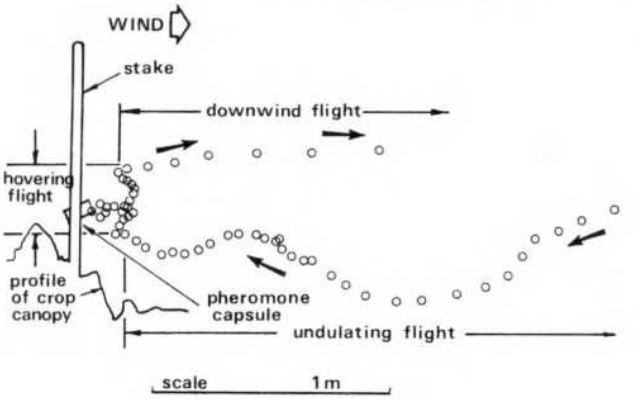
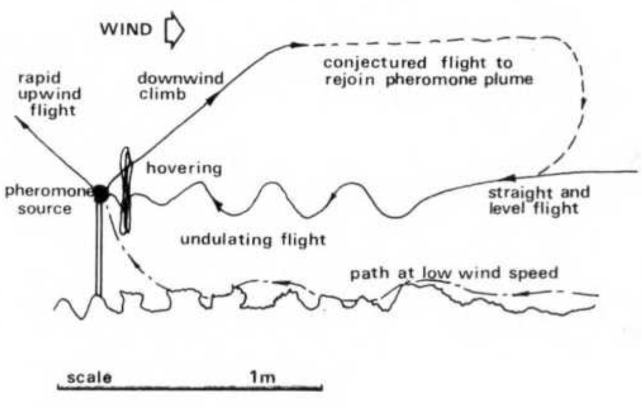


Fig. 2 Flight track produced from ciné film. The circles represent the moth's position on consecutive frames.

Closer to the source, forward progress was halted and vertical oscillations increased until the moth was hovering downwind from the source and moving up and down over a distance of up to 1 m while maintaining its upwind heading (Figs 1 and 2). Hovering could last up to 10 s during which occasional, less regular, short, downwind flights and general exploratory movements were made. About half of these moths then turned and flew rapidly downwind, often climbing to an altitude of order 2–3 m where they were usually lost from view. About half of them again landed near or on the capsule and moved about rapidly in a small area, often fanning with the wings. In Fig. 2 the moth very briefly touched the capsule before flying away in a gentle downwind climb accelerating to 2.2 m s^{-1} by the last frames. The moths which did land usually settled for up to about 1 min before leaving the capsule either downwind or in a rapid climbing upwind flight, again generally out of the illuminated region. On five nights moths were observed from a greater distance, 30–40 m, and it was then possible to see them climbing from the capsule and at an altitude of some few metres swiftly turning to head and fly downwind and then dropping to the original approach height about 10–20 m from the source. It was very difficult to see the moths after this because the image they produced in the NVD at such distance could be confused with

Fig. 3 Generalised flight pattern and the effect of low wind speed on it.



that produced by the lucerne leaves, but on two occasions it was possible to see them flying towards the attractant source again. At lower wind speeds the initial stages of the approach flight were made at very low altitude; in winds below about 0.1 m s^{-1} moths flew within the crop canopy, from which they made the final approach in a climb which became steeper as wind speed became less.

The observations suggest that the flight of male *Spodoptera littoralis* to a pheromone source is made in discrete stages with different flight patterns and perhaps also different orientation mechanisms. The flight paths observed showed a strongly vertical undulation pattern which contrasts with the horizontal zig-zagging more generally seen. This is not understood, but it may be due in some way to differences in the structure of the pheromone plume.

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Effect of regeneration on compartment specificity of the bithorax mutant of *Drosophila melanogaster*

IN insect imaginal disks the basis for determination may be the state of a series of loci, each switched on or off in a given cell lineage at a unique point in development known as a compartmentalisation event[1]. Clones induced after the blastoderm stage define homologous anterior and posterior compartments in the thoracic disks[2]. Bithorax mutants transform the anterior compartments of the metathoracic disks (haltere and third leg) into those of the mesothorax (wing and second leg); however, a wild-type allele must function continuously until metamorphosis to prevent the transformation[3,11]. This suggests that permanent activation of the gene at blastoderm accounts for heritability of the determined state. Compartmental commitments are apparently lost when disk fragments regenerate[4,5]. We therefore studied duplication patterns in the third legs of a temperature-sensitive cell-lethal mutant also homozygous for bithorax. Heat treatment of these larvae kills cells, producing disk fragments which regenerate *in situ* giving duplicated appendages[6–8]. We report here that bithorax sometimes transforms posterior compartments of duplicated legs, showing that its effects are not invariably compartment-specific.

Duplicated and deficient (DFP) third legs from heat-treated *726; bx^{34e}/MT6* or *726; bx^{34e}/bx^{34e}* individuals were scored for several morphological markers that differ between meso-and metathorax. 'Original' and 'duplicate' members of DFP legs could be distinguished on the basis of symmetry and size. Control data were collected by scoring unduplicated legs from the same flies and from a sample of bx^+ controls. The results are presented in Table 1. The bx^+ data demonstrate the reliability of the markers. The only marker that lacks complete specificity is the 'hairy island bristle' (BH^-). This is invariably present in mesothoracic legs, but also appears in the metathorax, especially in *TM6* heterozygotes. The data from bx^{34e} and bx^{34e}/*TM6* flies should therefore be compared only with the appropriate controls when this marker is considered.

Among bithorax third legs we found only markers of the mesothorax in the anterior compartments. In the posterior compartments of unduplicated legs and original members of DFPs we found only metathoracic markers (rows 5–8 of Table 1). But, in the posterior compartments of duplicate members we found markers of both meso- and metathorax (rows 9 and 10).

These results clearly show (1) that the effect of bithorax is confined to the anterior compartment of the unduplicated third leg, as in the haltere, (2) that the penetrance of the bx^{34e} allele is complete in the leg, and (3) that when the leg duplicates, the posterior compartment of the duplicate is sometimes also transformed. The transformation usually affects every marker in the compartment. Only 2 out of 50 cases are putative exceptions, each having a single discordant marker. Examples of the two major classes of duplication are illustrated in Fig. 1.

The posterior compartment of the duplicate was transformed to mesothorax in 18 out of 50 DFPs. The incidence of transformation was not significantly different in the two genotypes tested (Table 2*a*). The possibility that trans-

Table 1 Number of times markers of meso- and metathoracic legs were present in samples of duplicated third legs and in controls

Kind of leg	Genotype	Number	Markers												
			Anterior meso-				Posterior meta-				Posterior meso-				
			SP	*ThB*	*EB*	*AB*	*Y*	*1D*	*TR*	*BR*	*P*	BH^-	*2P*	*TSp*	*R2*
Normal 2nd	+ +	20	20	20	20	20	0	0	0	0	20	20	20	20	20
	+/*TM6*	20	20	20	20	20	0	0	0	0	20	19	20	20	20
Normal 3rd	+/+	20	0	0	0	0	20	10	20	20	0	2	0	0	0
	+/*TM6*	20	0	0	0	0	20	9	20	20	0	11	0	0	0
	bx^{34e}	40	40	40	40	40	40	8	40	40	0	0	0	0	0
	bx^{34e}/*TM6*	44	44	44	44	44	44	22	44	44	0	13	0	0	0
DFP 3rd (original)	bx^{34e}	31	29	29	31	31	26	2	30	31	0	0	0	0	0
	bx^{34e}/*TM6*	19	16	16	19	19	13	7	19	19	0	6	0	0	0
DFP 3rd (duplicate)	bx^{34e}	31	27	27	30	29	16	0	19	22	6	4	3	8	10
	bx^{34e}/*TM6*	19	16	16	17	17	9	1	10	10	3	5	3	7	7

Experimental flies were obtained by breeding from a *yvf726*; bx^{34e}/*TM6* stock. The symbol '*726*' refers to a temperature-sensitive lethal allele, *l(1)ts726*, at the suppressor-of-forked locus (1-65.9). When larvae carrying *726* are placed for 48 h at 29 °C, pattern deficiencies and duplications (DFPs) are formed in the surviving adults[6]. Cell-death creates disk fragments that duplicate during the extended larval period caused by heat treatment[7]. Leg deficiencies affect contiguous sets of markers located medially on the fate map[8]. bx^{34e} is an allele at the bithorad locus (3-58.8). The third chromosome balancer *TM6* carries the markers bx^{34e} and Ubx^{67b} (Ultrabithorax; 3-58.8) which is lethal as a homozygote. Further details may be found in Lindsley and Grell[9]. Homozygous bx^{34e} progeny were distinguished from bx^{34e}/*TM6* heterozygotes by scoring the halteres which are much more strongly transformed when *TM6* is present. Parents were allowed to lay eggs for 5 d at 22 °C in bottles containing a standard medium[6]. The parents were discarded and the cultures shifted to 29 °C for 48 h then returned to 22 °C. On eclosion, adults of appropriate genotypes were collected and examined for duplications. Pharate adults that failed to eclose were dissected from the pupal case and treated similarly. Flies with duplications were dissected and mounted for detailed examination as described elsewhere[8]. Morphological markers were scored under the compound miscroscope. For details of markers used see Fig. 1 legend. Markers were assigned to compartment following Steiner (1976)[2].

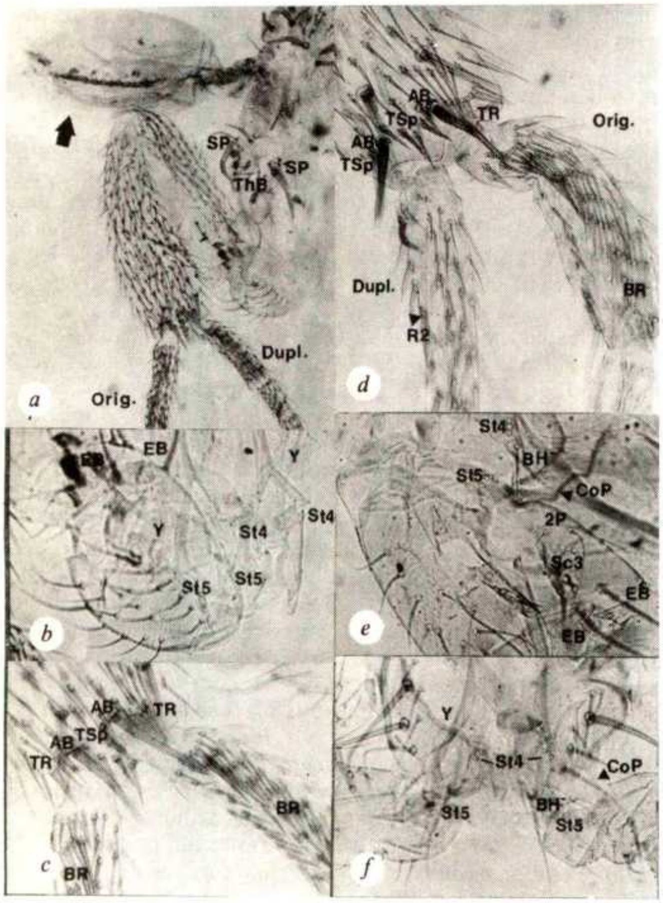


Fig. 1 *a–c*, DFP third leg in a $bx^{34e}/TM6$ fly; *b* and *c* show details of markers in proximal and distal segments. Anterior haltere is transformed into wing (arrow). Anterior compartments of both original and duplicate parts of leg bear mesothoracic markers: sternopleurals (SP), thoracic bristles (ThB), edge bristles (EB) and apical bristles (AB). The posterior compartments of both elements bear the following metathoracic markers: the Y-shaped apodeme (Y), tibial transverse row (TR), basitarsal transverse rows (BR). *d–e*, DFP bx^{34e} third leg. The duplicate posterior compartment is transformed into that of the second leg. In *d* note tibial spurs (Tsp) flanking the apical bristle on both sides in the duplicate, and on one side in the original. Duplicate also has a single row of bristles (R2) in the basitarsus where the original has transverse rows (BR). Coxa and trochanter are shown in *e*. Markers in the focal plane belong to the duplicate posterior compartment, and are characteristic of the mesothoracic leg. BH^-, hairy island bristle; CoP, coxal process; 2P, bristles proximal to Sc3. St4, St5 and Sc3 are found in both meso- and metathoracic legs (compare Fig. 1*b*). *f*, DFP 3rd leg in which duplicate is mesothoracic. This example shows the Y-apodeme in the original leg (Y) with arms adjoining St4 and St5. This clearly is not present in the duplicate, which instead shows a hairy island bristle (BH^-) and a coxal process (CoP).

formation depends on stage at heat treatment is ruled out by the fact that in four out of five cases in which both third legs from the same fly were duplicated, one was transformed, and the other was not. We also scored each DFP for a series of morphological markers common to meso- and metathoracic legs. Deficiencies for these markers may either fall entirely within the anterior compartment, or may also extend into the posterior[8]. Posterior cells might be expected to participate more often in the formation of the regeneration blastema in the latter event. Contrary to expectation, no significant difference in the frequency of transformation was found (Table 2*b*).

Table 2 Incidence of transformation of posterior compartment of duplicate, classified by *a*, genotype and *b*, extent of deficiency

	a Genotype		*b* Deficiency	
	bx^{34e}	$bx^{34e}/TM6$	Anterior only	Anterior and Posterior
Transformation				
Anterior only	21	11	21	11
Anterior + posterior	10	8	13	5
Contingency χ^2	0.49		0.23	

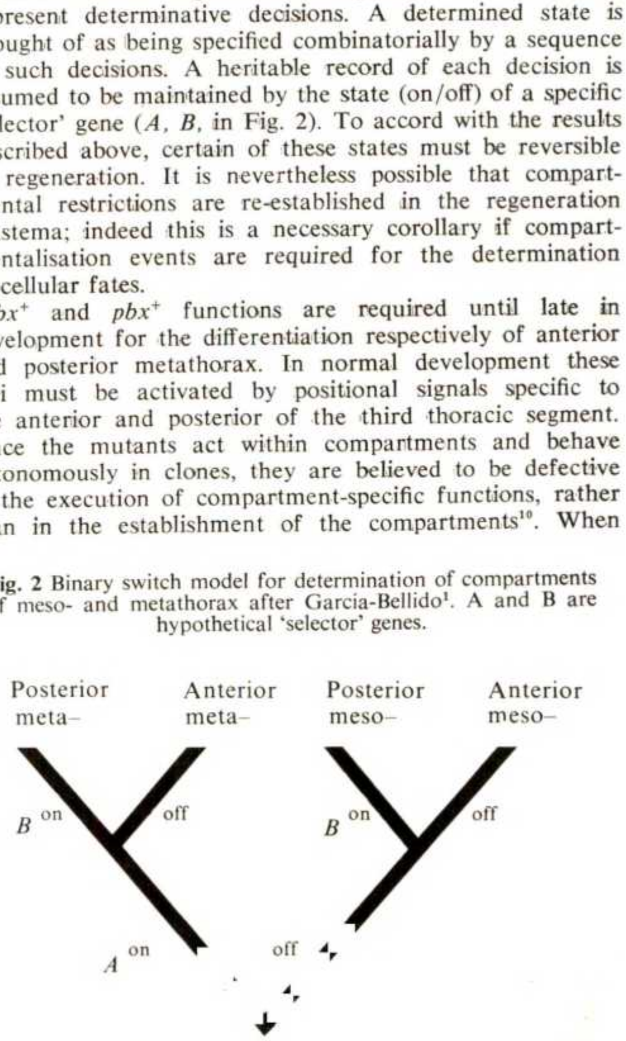
The following interpretation is tentative, but susceptible of verification. Transformation of the duplicate posterior compartment implies that the regeneration blastema is sometimes derived from anterior cells in the original leg. Schubiger[4] showed that anterior fragments[2] can regenerate an entire leg, and Haynie and Bryant[5] have confirmed that regeneration across compartment boundaries can occur. In bithorax homozygotes, anterior cells normally develop as mesothorax. Our results suggest the transformed state may be maintained when these cells form the posterior compartment of the duplicate.

Classification of DFPs by size of deficiency provides no support for the idea that regeneration of a metathoracic posterior compartment depends similarly on recruitment of posterior cells from the original leg. Although we have no direct evidence we prefer the alternative hypothesis which assumes that anterior cells have some finite chance of changing state when they enter the regeneration blastema. The work of Adler (personal communication) provides direct support for this interpretation. Anterior fragments of bx^3 haltere disks were occasionally able to form posterior haltere when stimulated to regenerate across the compartment boundary.

Garcia-Bellido[1] argues that compartmentalisation events represent determinative decisions. A determined state is thought of as being specified combinatorially by a sequence of such decisions. A heritable record of each decision is assumed to be maintained by the state (on/off) of a specific 'selector' gene (*A*, *B*, in Fig. 2). To accord with the results described above, certain of these states must be reversible in regeneration. It is nevertheless possible that compartmental restrictions are re-established in the regeneration blastema; indeed this is a necessary corollary if compartmentalisation events are required for the determination of cellular fates.

bx^+ and pbx^+ functions are required until late in development for the differentiation respectively of anterior and posterior metathorax. In normal development these loci must be activated by positional signals specific to the anterior and posterior of the third thoracic segment. Since the mutants act within compartments and behave autonomously in clones, they are believed to be defective in the execution of compartment-specific functions, rather than in the establishment of the compartments[10]. When

Fig. 2 Binary switch model for determination of compartments of meso- and metathorax after Garcia-Bellido[1]. A and B are hypothetical 'selector' genes.



bx^+ pbx^+ third legs duplicate, the posterior compartment is always metathoracic. In the context of the model this implies activation of pbx^+ and thus, heritability of the decision that distinguishes meso- from metathorax. Transformation of the duplicate posterior compartment when the genotype is bx^- pbx^+ therefore implies that bx^+ function is necessary for heritability of this decision. If a memory of the determinative event existed in the state of an independent selector gene (A in Fig. 2), we would not expect a mutation at bithorax to influence activation of postbithorax. Supported by NRC (Canada) grant number A6485 to M.A.R.

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Application of magnetic microspheres in labelling and separation of cells

POLYMERIC microspheres conjugated to antibodies and lectins have been used previously as cell surface markers for the detection and localisation of antigens and lectin receptors using scanning electron microscopy (SEM) (refs 1–4). In this report we describe a new, more versatile cell surface probe consisting of iron-containing polymeric microspheres tagged with fluorescent dyes and chemically coupled to antibodies or lectins. The magnetic and fluorescent properties of these microspheres have been utilised in the magnetic separation of red blood cells (RBC) and lymphoid cells and in the detection of immunoglobulin (Ig) receptors and wheat germ agglutinin (WGA) receptors on lymphocytes and Hela cells by SEM and fluorescent microscopy.

Magnetic, polymeric microspheres were synthesised from iron oxide (Fe_3O_4) particles (Ferrofluidics, Burlington, Massachusetts, No. A01, 5%, w/v) and from methyl methacrylate (MMA), hydroxethyl methacrylate (HEMA), methacrylic acid (MAA), and ethylene glycol dimethacrylate (EGDA) purified as described previously[1,2]. Briefly, a mixture of 1.6 g MMA, 0.9 g HEMA, 0.3 g MAA, 0.2 g EGDA, 1 g iron oxide suspension and 1 g Triton X-405 was de-aerated and polymerised by Co γ irradiation (0.2 Mrad at 0 °C for 60 min)[5]. The Fe microsphere suspension was purified on a mixed-bed ion-exchange column[1] and centrifuged at 30,000*g* for 45 min at 4 °C on a discontinuous gradient consisting of 20% (w/w) sucrose in the upper layer and 60% (w/w) in the lower layer. Three fractions (brown in colour) were recovered: an upper band in the 20% sucrose fraction (Fe content 6.2%); a lower band in the 20% sucrose layer (Fe content 28.4%); and a pellet (Fe content 42.4%). The upper and lower fractions were used in cell labelling studies. The average size of these particles was 40 nm as measured by SEM.

Copolymer Fe microspheres were derivatised with diaminoheptane by the cyanogen bromide method[1,6] and tagged with 1 mg fluorescein isothiocyanate (FITC) per ml suspension in carbonate buffer, *p*H 9.5, for 12 h at 25 °C. After removal of excess FITC, the spheres were re-derivatised with diaminoheptane using the carbodiimide method[1].

Affinity purified antibodies or lectins were coupled to the fluorescent Fe microspheres by the two-step glutaraldehyde reaction[1,7]. A protein concentration of 0.5–1.0 mg ml^{-1} and a Fe microsphere concentration of 1 mg ml^{-1} was used in the coupling stage. The reaction was carried out for 5–12 h at 25 °C. Unbound protein was separated from the protein–microsphere conjugate either by centrifugation on a discontinuous sucrose gradient[1] or by chromatography on Sepharose 6B. The conjugates could be concentrated with a magnet placed against the wall of a glass vial.

The application of magnetic immunomicrospheres in the separation of cells was tested in two independent systems. In the first, glutaraldehyde-fixed mouse thymocytes indirectly labelled for surface antigens with fluorescent–Fe microsphere–antibody conjugates were mixed in varying proportions with unlabelled human RBC. The mixture was layered over phosphate-buffered saline (PBS) containing 5% bovine serum albumin (BSA) and placed in a magnetic field (horseshoe magnet, 12 lb pull). After 2 h the solution was eluted to separate cells attracted to the magnet from those that were not. Differences in cell morphology and fluorescent labelling were used to analyse the cell fractions. The rate of deflection of labelled cells towards the magnet depended on the extent of labelling and the Fe content of the microspheres. As indicated in Table 1, over 99% of the labelled thymocytes were attracted by the magnet.

In a more applicable system, this technique was used to separate out lymphocytes displaying Ig receptors (B cells) from a mixed population of spleen lymphocytes. Fluorescent Fe microspheres coupled to goat anti-mouse Ig were used to directly label unfixed lymphocytes. Labelled cells could be readily distinguished from unlabelled cells under a fluorescent microscope (Fig. 1). In agreement with literature values[8,9] approximately 37% of the cells were labelled (Table 1). The mixture of labelled and unlabelled cells was subjected to a magnetic field. Previous removal of unbound Fe microspheres was not necessary. After 2 h at 4 °C, 97–99% of the labelled cells (principally B lymphocytes) were retained by the magnet, leaving a highly purified population of unlabelled lymphocytes (T cells). Some unlabelled cells, however, were also in the cell population which was retained by the magnet. This resulted largely from nonspecific aggregation of the lymphocytes and nonspecific adhesion of cells to the glass wall of the pipette. Further removal of unlabelled cells from the predominantly labelled cell population could be achieved by a second passage of dissociated cells through the magnetic field. A detailed study of optimal conditions for cell separation will be given elsewhere.

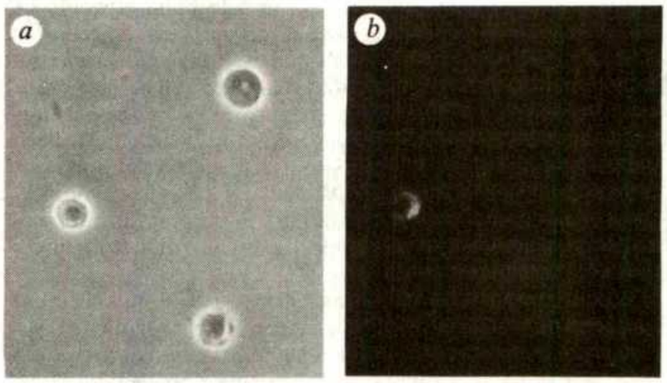

Fig. 1 Mouse spleen lymphocytes directly labelled for surface immunoglobulin (Ig) receptors with fluorescein–Fe microsphere-goat anti-mouse Ig antibody conjugates. *a*, Lymphocytes viewed by phase contrast light microscopy. *b*, The same lymphocytes viewed by fluorescent microscopy. Only one lymphocyte (B cell) is labelled with the conjugates.

Application of these fluorescent Fe-microspheres as visual markers for SEM was also demonstrated. Figure 2*a* illustrates the labelling of wheat germ agglutinin receptors on cultured Hela cells. Specificity was verified by the absence of binding of the

Table 1 Magnetic cell separation

Experiment	Before separation No. of cells counted Total	 Labelled (Fl)*	% Labelled (Fl)	Magnet	After separation No. of cells counted Total	 Labelled (Fl)*	%Labelled (Fl)
1 (RBC/thymocytes)	521	429	82.3	Attracted	509	499	98.0
				Not attracted	500	2	0.4
2 RBC/thymocytes	580	179	30.9	Attracted	532	487	91.5
				Not attracted	520	1	0.2
3 (lymphocytes)	252	92	36.5	Attracted	206	168	81.6
				Not attracted	211	3	1.4
4 (lymphocytes)	210	82	39.1	Attracted	144	110	76.4
				Not attracted	303	9	3.0

*Labelled with fluorescein–Fe microsphere–antibody conjugates.

In RBC-thymocyte experiment 1, 2.1×10^6 glutaraldehyde-fixed RBC were mixed with 8.2×10^6 fixed mouse thymocytes labelled sequentially with rabbit anti-thymocyte antiserum followed by fluorescein–Fe microsphere–goat anti-rabbit immunoglobulin conjugates. Experiment 2 was carried out in similar conditions, except that 8.1×10^6 RBC were mixed with 3.3×10^6 thymocytes. The number of fluorescent cells was measured. Approximately, 5×10^6 mixed cells were layered on a 5% BSA–PBS solution. A magnet (12 lb pull) was placed against the wall of a 0.9-cm diameter column at the interface. After 2 h at 4 °C the column was gently eluted with PBS to separate cells attracted to the magnet from those which were not. The magnet was then removed from the side of the column, and the cells pulled to the side of the column were displaced by shaking the column. The two cell fractions were analysed for fluorescent (Fl) labelling and for cell type using a Leitz Dialux fluorescent microscope. In experiments 3 and 4, 1×10^6 mouse spleen lymphocytes purified by the Ficoll isopaque method[13] were directly labelled with 0.1 ml fluorescein–Fe microsphere–goat antimouse Ig conjugates at 4 °C. Cells were washed and the percentage of cells with fluorescein label was measured. Approximately 5×10^5 cells were then layered on a Ficoll isopaque layer and subjected to magnetic separation as described above.

WGA–microsphere conjugate in the presence of *N*-acetylchitobiose, an inhibitor of WGA (Fig. 2*b*).

These new cell surface reagents have potential applications in biochemical and microscopic studies of specific components on cell surface membranes. The magnetic properties of these spheres can be utilised to isolate specific types of cells. The magnetic cell sorting technique described here is quite simple, but more sophisticated instrumentation can be envisioned to continuously separate cells which differ in the number, as well as the nature of surface molecules. Such techniques would be particularly useful for processing large numbers of cells for biochemical and immunological studies. Magnetic properties can also be used in the separation of labelled cell surface membranes from intracellular membranes, as well as in the purification of specific membrane-bound receptors which have been solubilised in mild detergents. Stronger magnetic fields may be required in such applications. Alternatively, the dense properties of these spheres can be exploited to separate membranes and receptors by density or velocity perturbation techniques using ultracentrifugation[10,11].

The iron content of these microspheres also permits their use as visual markers for transmission electron microscopy (TEM). This enables one to correlate labelling information derived from light and SEM with cellular ultrastructure obtained by TEM. In principle, similar reactions used in the synthesis of these iron-containing polymeric spheres can be adapted for preparing microspheres containing gold or other heavy metals which have advantages for SEM and TEM (refs 4, 12).

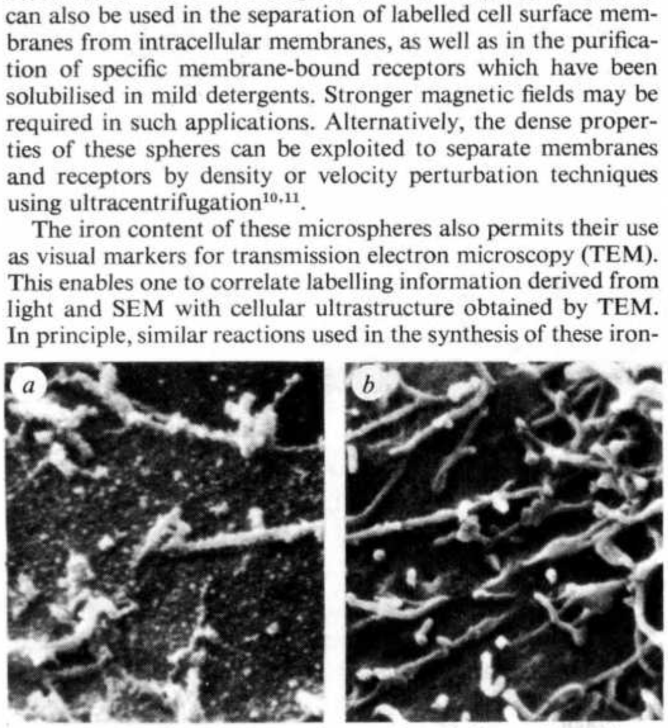

Fig. 2 Scanning electron micrographs of the surface of a cultured Hela cell labelled with fluorescein–Fe microsphere–WGA conjugates in *a*, the absence and *b*, the presence of the WGA inhibitor, *N*-acetyl chitobiose. Microspheres bind only when the inhibitor is absent during labelling.

Finally, these or related reagents may have future clinical applications. Magnetic microspheres carrying cytotoxic drugs, enzymes or radioisotopes could, in principle, be localised in a certain tissue of a body such as a tumour, by applying powerful magnets and serve as diagnostic or therapeutic agents.

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Formation and resealing of pores of controlled sizes in human erythrocyte membrane

APPLICATION of an electric pulse, at field intensities of a few kV cm^{-1} and of duration in the μs range, to an isotonic suspension of erythrocytes is known to cause haemolysis of the red cells[1–4]. Studies from different laboratories suggest that the haemolysis is due to the field-induced transmembrane potential[1,3,4]. Our recent experiments[5] indicate that once the transmembrane potential reaches a threshold of approximately 1 V, which corresponds to an applied field of 2.2 kV cm^{-1}, the erythrocyte membrane becomes leaky to normally impermeant ions or molecules. The permeation of solutes leads to the swelling and eventual lysis of the red cells. This type of haemolysis is known as colloid osmotic haemolysis[6,7]. The voltage-induced permeability change is consistent with the formation of pores in the membrane. We show here that the size of these pores can be varied in a controlled manner, and that the leaky membrane can be resealed while the haemolysis is prevented. Foreign molecules have successfully been incorporated into the resealed, but otherwise intact, erythrocytes.

In the experiment shown in Fig. 1, erythrocytes in an isotonic NaCl solution were treated with a 3.7 kV cm^{-1}, 20-µs pulse, and the subsequent change in cell volume V was monitored by light scattering measurement. In these conditions, intracellular K$^+$ leaks out, being replaced with Na$^+$, within a few minutes. Further entry of NaCl causes the swelling of the red cells[5]. When V reached 1.1 V_0 (where V_0 is the volume of the untreated cells) a small amount of isotonic solution of various substances was added to the suspension (time $t = 0$). Curve *d* in Fig. 1 is a control, where 1/10 volume of isotonic NaCl was added. The cells kept swelling because of the continuous influx of NaCl. However, when the same amount of sucrose solution was added, the swelling immediately stopped as shown in curve *b*. A separate tracer experiment showed that the cell membrane is practically impermeable to sucrose in these conditions. Therefore, the above result indicates that the reduction of the external NaCl concentration by 9% is just sufficient to abolish the electrochemical gradient, or the resultant uptake, of NaCl. As expected, the addition of a larger amount of sucrose solution reduced the external NaCl concentration further, and the cells began to shrink due to the efflux of NaCl (curve *a*).

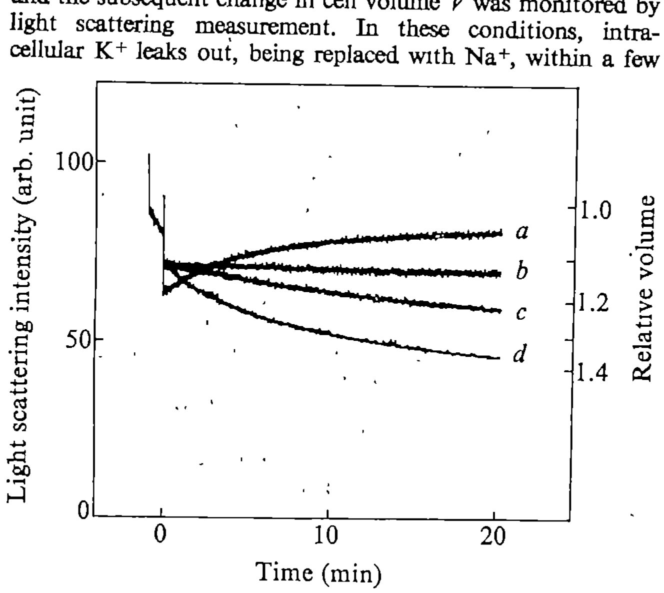


Fig. 1 Changes in cell volume after the electric pulsation. Washed human erythrocytes were suspended in an isotonic NaCl solution (150 mM NaCl, 7 mM phosphate buffer, *p*H 7 0) at a volume concentration of 1%, and treated with a single square-wave electric pulse of intensity 3.7 kV cm^{-1}, duration 20 µs, between a pair of stainless steel electrodes After 10–20 s, an aliquot was taken and mixed with 50 volumes of the isotonic NaCl solution, and the change in light scattering intensity at 600 nm was recorded with an Aminco–Bowman spectrofluorometer equipped with a magnetic stirrer. The scattering intensity was calibrated against the cell volume V by a separate haematocrit measurement, as shown in the right-hand scale which applies to the $t > 0$ portion of curves *b*–*d*. When V reached 1.1 times that of the untreated V_0, the following additions were made ($t = 0$). Curve *a*, 2/10 volume of isotonic (272 mM) sucrose solution to 1 volume of the suspension; curve *b*, 1/10 volume of the isotonic sucrose solution; curve *c*, 1/10 volume of isotonic (285 mM) xylitol solution; curve *d*, 1/10 volume of the isotonic NaCl solution. Temperature was 25 °C. In these conditions, the extent of haemolysis is negligible for the 20-min period shown; however, all the cells haemolyse by 15 h unless the carbohydrates are added.

When the added substance was xylitol (MW 152 whereas sucrose is 342), only partial blocking of the cell swelling was achieved as shown in curve *c*. The volume change in this situation is expected to satisfy the following relation

$$\mathrm{d}V/\mathrm{d}t = (j_x + j_{\mathrm{NaCl}})V/C \qquad (1)$$

where j_x and j_{NaCl} are the net influxes of xylitol and NaCl respectively, in mOsmol l^{-1} cells h^{-1} and C the total osmotic concentration of the medium in mOsmol l^{-1}. At $t \approx 0$ or $V \approx V_0$, the net influx j_{NaCl} is negligible, as shown in curve *b*, and the swelling is almost entirely due to the xylitol influx j_x. Thus the rate of permeation of xylitol molecule k_x can be roughly estimated as

$$k'_x \cong j_x/C_x = (V^{-1}\mathrm{d}V/\mathrm{d}t)_{t=0}(C/C_x) \qquad (2)$$

where C_x is the external osmotic concentration of xylitol ($C/C_x = 11$).

In this way, the permeability of the pulse-treated membrane to 11 different carbohydrates was measured in various conditions. (Tracer influx measurements on a few selected carbohydrates gave values consistent with those obtained by the above method.) The results are plotted in Fig. 2 against the average radius of the tested molecule. Curve *a* gives data for the untreated cells; molecules larger than erythritol do not enter the cells to any appreciable amount, except D-glucose which is known to be carried by a specific transport system[7]. This agrees with the idea that pores of a radius of about 3.5–4.2 Å exist in human erythrocyte membrane[8]. Curve *b* shows

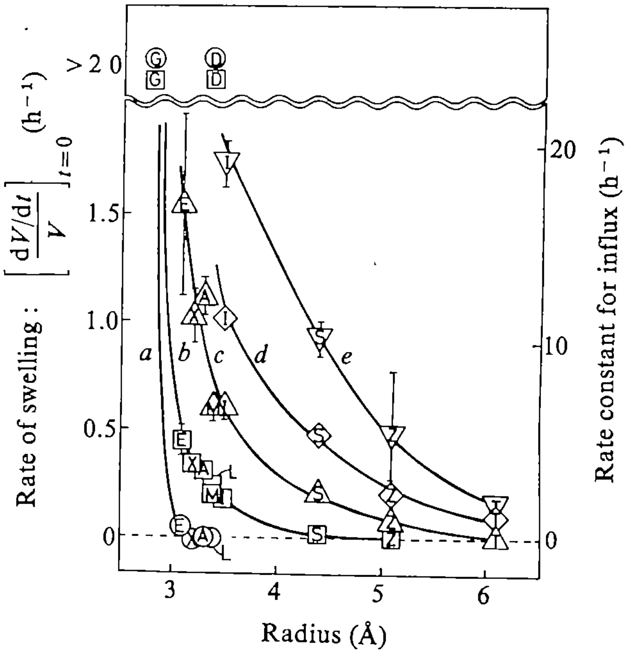


Fig. 2 Permeability of the pulse-treated erythrocyte membrane to various carbohydrate molecules. Curve *a*, untreated cells; curve *b*, cells treated with a 3.7 kV cm^{-1}, 20-µs pulse in isotonic NaCl solution; curve *c*, 5.3 kV cm^{-1}, 20-µs, in NaCl; curve *d*, 3.7 kV cm^{-1}, 80 µs, in NaCl; curve *e*, 3.7 kV cm^{-1}, 20 µs, in a 3:7 mixture of isotonic NaCl and isotonic sucrose solutions. After the pulsation, swelling of the cells due to the influx of various carbohydrate molecules was recorded as in curve *c* of Fig. 1 The rate of swelling was determined from the initial slope of the swelling curve; the rate of permeation was calculated from equation (2). The average radius in the abscissa denotes $(r_1 r_2 r_3)^{1/3}$ where the r_i values are three orthogonal radii measured in space-filling molecular models. G, glycerol; E, meso-erythritol; X, xylitol; A, D-arabitol; D, D-glucose; L, L-glucose; M, D-mannitol; I, myo-inositol; S, sucrose; Z, melezitose; T, stachyose.

Table 1 Incorporation of sucrose in resealed erythrocytes

Procedure		Assay					
Treatment	Medium	Time	% survival	Relative cell volume	Sucrose content (mmol l^{-1} cells)	Na^+ content (mmol l^{-1} cells)	K^+ content (mmol l^{-1} cells)
a Pulsation 3.7 kV cm^{-1}, 80 μs, 25 °C	NaCl 135 mM, NaP* 6 mM, sucrose 27 mM, *p*H 7.0	before *a*	100	1.00	0.0	91	10
b Resealing 1 37 °C, 1 h	NaCl 119 mM, NaP 7 mM, sucrose 27 mM, stachyose 21 mM, $MgSO_4$ 2 mM, *p*H 7.2	after *b*	99	0.99	3.2	92	11
c Resealing 2 37 °C, 6 h	NaCl 107 mM, KCl 5 mM, sucrose 27 mM, stachyose 21 mM, adenosine 3 mM, inosine 1 mM, supplement†, *p*H 7.5	after *c*	97	0.94	3.5	98	17
d Resealing 3 37 °C, 12 h	NaCl 107 mM, KCl 5 mM, $CaCl_2$ 2 mM, stachyose 40 mM, adenosine 0.3 mM, inosine 0.1 mM, supplement, *p*H 7.5	after *d*	95	1.01	3.2	81	27
e Test incubation 37 °C, 72 h	NaCl 133 mM, KCl 5 mM, $CaCl_2$ 2 mM, adenosine 0.15 mM, inosine 0.05 mM, supplement, *p*H 7.5	24 h in *e*	74	1.00	3.3	63	42
		48 h in *e*	65	0.99	3.3	55	46
		72 h in *e*	58	0.95	3.2	—	—

* NaP, Sodium phosphate buffer.

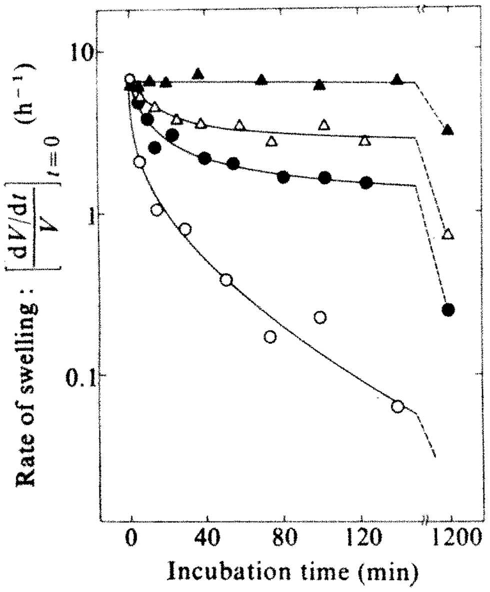
† Supplement: NaP 7 mM, $MgSO_4$ 2 mM, D-glucose 11 mM, chloramphenicol 0.1 mg ml^{-1}, penicillin G 500 units ml^{-1}, bovine serum albumin 30 mg ml^{-1}. Sucrose contained trace amount of [^{14}C] sucrose.

Volume concentration of the cells was ~ 20% at stage *a*, 10% at *b*, 3–5% at *c* through *e*. The suspension was gently rocked during the incubations. In *e*, medium was changed at every 12 h, during which *p*H dropped by 0.2–0.3 unit. Percentage survival was calculated from the amount of haemoglobin released in the supernatant. Relative cell volume was assumed to be inversely proportional to the amount of haemoglobin contained in a unit volume of packed cells (haemoglobin content of the untreated cells was 315±6 g l^{-1} cells). Sucrose content was determined from the radioactivity of the cells packed in microhaematocrit tubes. Na^+ and K^+ were assayed by flame photometry. Assay values except % survival refer to survived (unlysed) cells. All values are averages of several determinations on two independent samples; variations were within ±1% for % survival, ±0.02 for cell volume, ±0.2 mmol l^{-1} cells for sucrose, ±5 mEq l^{-1} cells for Na^+ and K^+.

that the treatment with a 3.7 kV cm^{-1}, 20, μs pulse increases the critical size for permeation: molecules smaller than sucrose can penetrate the membrane, and the rate decreases with the size of the molecule. Again, an exception to this curve is D-glucose; the specific transport system seems to be intact even after the pulse treatment. As can be seen from curves *c*, *d* and *e*, larger pores are obtained either by using a higher field intensity, by increasing the pulse duration, or by reducing the ionic strength of the pulsation medium.

As suggested by curve *b* in Fig. 1, addition of a sufficient amount of impermeant substance to the suspension of pulse-treated erythrocytes retards the haemolysis indefinitely. While the cells are prevented from lysis, the membrane spontaneously reseals as in the case of ghosts obtained by hypotonic lysis[9]. The resealing process is strongly temperature-dependent, as shown in Fig. 3. The ordinate is the rate of swelling measured in isotonic NaCl; as before, this rate is proportional to the net influx of NaCl. At 37 °C the treated membrane rapidly regains its impermeability to cations, whereas at 3 °C the cells remain highly permeable even after 20 h.

Fig. 3 Time courses of the resealing of pulse-treated erythrocytes at different temperatures. ▲, 3 °C; △, 17 °C; ●, 25 °C; ○, 37 °C. The washed erythrocytes in isotonic NaCl were treated with a 3.7 kV cm^{-1}, 80-μs pulse at 25 °C, and immediately mixed with a large volume of 85:15 mixture of isotonic NaCl and isotonic stachyose solutions kept at the specified temperatures. At intervals, an aliquot was taken and added to an isotonic NaCl solution of the same temperature, and the initial rate of swelling was measured as described in Fig. 1. Haemolysis in the incubation media (NaCl–stachyose) was negligible except at 3 °C, where a small portion of the cells haemolysed by 20 h presumably because of the very slow penetration of stachyose.



The electric pulsation followed by an appropriate resealing procedure makes it possible to prepare erythrocytes (not ghosts) with altered intracellular compositions. For example, the ionic composition of the resealed cells reflects that of the resealing medium: incubation in NaCl or KCl media yields cells loaded predominantly with Na^+ or K^+ respectively. Although the alteration of cellular cations can also be achieved by lactose treatment[10] or chemical modification of the cell membrane[11], the present method allows the incorporation of larger molecules such as sucrose by introducing pores of adequate size. Results of a typical experiment are shown in Table 1. The intactness of the loaded cells was tested by incubation for an additional 72 h in a simulated physiological medium at 37 °C. Although some haemolysis occurred, especially during the first 24 h of the test incubation, about 60% of the original cells survived the 3-d period. The cells in the medium assumed either normal biconcave or slightly cup-shaped disk forms and maintained approximately normal cell volume. The resealed erythrocytes accumulated K^+ and extruded Na^+ against a concentration gradient, indicating that the pores were almost completely annealed and the Na–K pump was intact.

At least two applications of the present technique are conceivable: (1) the alteration of intracellular compositions will simplify experimental designs, especially in transport studies. (2) Erythrocytes may be used as intravenous drug reservoirs; gradual release from loaded erythrocytes could help maintain the drug level in a patient's circulation.

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Activation of mouse spleen cells by a single short pulse of mitogen

THE stimulation of lymphocytes by polyclonal mitogens such as concanavalin A (con A) is a useful system for analysing the activation of cells out of the resting state, G_0, into the division cycle. Optimal stimulation of the lymphocytes with con A, however, requires presence of the mitogen for about 20 h (ref. 1), and the cells transfer from G_0 into G_1 in an unsynchronised manner[2]. This lack of synchrony makes biochemical analysis of activation complicated because sequential events of the cell cycle become superimposed. I show here that a single short pulse of con A will irreversibly commit a significant proportion of murine spleen cells into the division cycle. This relatively synchronised stimulation of cells enables the requirements for activation to be analysed separately from subsequent events as the cells progress through the cell cycle. For example, it has been shown that the presence of colchicine during a 2-h exposure to con A inhibits mitogenic activation.

Spleen cells from 6–10-week-old male BALB/c mice were cultured in the absence of serum (Fig. 1 legend), and mitogenesis was measured by the incorporation of ^{3}H-thymidine into cellular DNA and the stimulation index (SI) calculated as c.p.m. con A-treated cultures/c.p.m. control cultures. Con A (1 μg ml^{-1}) was reduced to non-mitogenic levels after 3 h by changing the growth medium to remove free con A and adding 5 mM α-methyl-D-mannoside (α-MM) to displace cell-bound con A; this ratio of sugar to lectin (that is, 5 mM: 1 μg ml^{-1}) was used experimentally. The concentration of con A required for maximal mitogenic stimulation was dependent on the duration of exposure to the lectin (Fig. 1). Cells continuously exposed to con A gave a very sharp dose–response curve at 52 h which peaked at 1 μg ml^{-1} with SI = 252, and fell to background levels of DNA synthesis above 3 μg ml^{-1} con A. In contrast, identical cells given a 3-h pulse of con A showed a broad response curve peaking at 2–3 μg ml^{-1} at both 28 h and (SI = 25) and 52 h (SI = 183). This change in the dose–response curve is not a result of a change in the optimal dose requirements during the development of the mitogenic response, since the peak position was constant at both 28 h and 52 h following exposure to con A for either 3 h or 24 h (Fig. 1). It is relevant to note that when the dose–response curves were repeated in identical conditions, but using BALB/c thymocytes instead of spleen cells, 3-h exposure to con A did not produce a significant mitogenic response (maximal s.e. = 5.4 at 1.25μg ml^{-1} con A) although the same cells responded well after prolonged exposure to the lectin (Fig. 2).

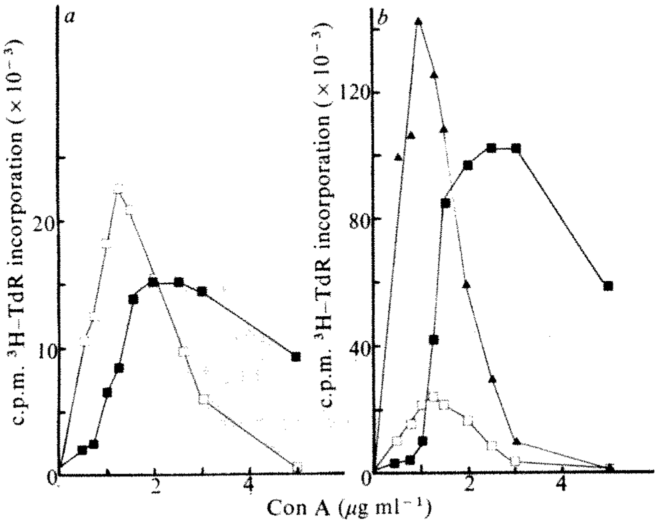

Fig. 1 Dose–response curves of BALB/c spleen cells to con A after 3-h, 28-h and 52-h exposure to the lectin. Labelling for *a*, 23–28 h; *b*, 47–52 h. Washed spleen cells from a single mouse were diluted to 5×10^6 ml^{-1} cells with growth medium (RPMI 1640 plus 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin) containing the required concentration of con A. Aliquots (100 μl) of these cells were dispensed into flat-bottomed wells of a microtitre tray and cultured in a humidified atmosphere of 10% CO_2, 7% O_2 and 83% N at 37 °C. Con A was removed by complete replacement of the supernatant with prewarmed growth medium containing the appropriate concentration of α-MM (see text). DNA synthesis was measured using standard techniques after labelling each culture with 0.5 μCi 6-^{3}H-thymidine (specific activity = 2.0 Ci mmol^{-1}) for 5 h. Each point represents the mean of 5 replicate cultures. ■, Con A 0–3 h; □, con A 0–28 h; ▲, con A 0–52 h. The same results were obtained in three separate experiments.

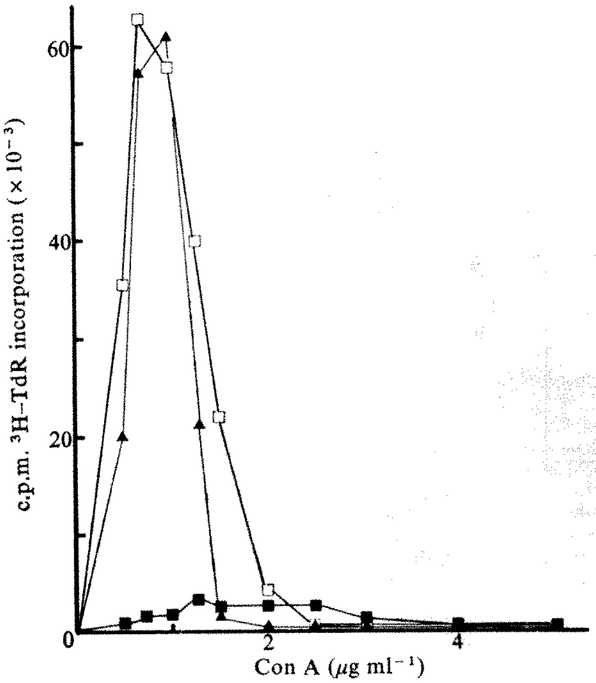

Fig. 2 Dose–response curves of BALB/c thymocytes to con A after 3 h, 28 h and 52 h exposure to the lectin. Experimental details as in the legend to Fig. 1. DNA synthesis was measured between 47–52 h. ■, Con A 0–3 h; □, con A 0–28 h; ▲, con A 0–52 h.

This time-dependent shift in the dose–response curve of spleen cells may be caused by a variety of cellular responses to con A which develop at different rates and show different sensitivity to the lectin. For example, concentrations of con A high enough to induce a complete activation signal in a short time may thereafter initiate an inhibitory effect dependent on prolonged exposure of the cells to con A. Lower concentrations of con A, although less effective during the inhibitory phase, require longer to generate a complete activation signal. A similar explanation has recently been suggested by McClain and Edelman[3]. On the other hand, the possibility that α-MM failed to reduce bound con A to non-mitogenic levels must be considered, although this is unlikely since addition of α-MM abolished

Table 1

Experiment no.	Time (h) 0 1 2 3	H^3-TdR incorporation (c.p.m.)	$b/a \times 100$
1 *a*		169+/−14	105%
b	←———C———→	178+/−33	
2 *a*	←———con A———→	75,142+/−6,927	87.3%
b	←—C—→←———con A———→	67,607+/−4,677	
3 *a*	←———con A———→	28,182+/−1,300	38.6%
b	←———con A+C———→	10,880+/− 908	
4 *a*	←———con A———→	43,482+/−1,727	93%
b	←———con A———→←—C—→	40,577+/−5,556	
	Time (h) 0 2 24 52		
5 *a*	←———con A———→	16,863+/− 724	26.8%
b	←———con A———→ ←—C—→	4,532+/− 322	

Murine spleen cells were cultured as described in the legend to Fig. 1. Cultures were exposed to 5 μg ml^{-1} con A and 0.5 μM colchicine (C) during the times indicated by the horizontal bars, and these agents were removed by complete replacement of the supernatant with prewarmed growth medium containing 25 mM α-MM. Controls were always treated identically to experimental cultures to ensure that the effect of medium change and sugar addition did not interfere with the results. ^{3}H-thymidine uptake was measured between 47–52 h and each value represents the mean of five cultures. In each experimental group *a* were the control cultures.

mitogenesis in control experiments. In addition, the different characteristics of cell populations responding to acute and prolonged exposure to con A (that is, the shape of the dose–response curves and duration of DNA synthesis) show that the α-MM did not cause a simple shift in the dose–response curve. Further confirmation of the efficacy of α-MM is apparent in the experiments with thymocytes, which responded well to prolonged exposure to con A but were unable to respond to a 3-h pulse.

These results are pertinent to the analysis of early cellular responses to con A which constitute an activation signal. Such analysis often uses con A concentrations which give maximal DNA synthesis at 48 h in the continuous presence of con A, but it is clear that this concentration of con A is insufficient to generate a significant activation signal within a short time. In contrast, short pulses of high concentrations of con A can activate cells within a few hours (Fig. 1). From these results it is suggested that in order to analyse the early events in the activation of cells from G_0 to G_1 it may be important to use a concentration of con A high enough to generate an activation signal within a short period.

The finding that lymphocytes will transform after a single short pulse of con A contrasts with results of Toyoshima *et al.*[4], who found that two 3-h pulses, separated by a gap of 12 h, are necessary for optimal con A stimulation (SI = 3.9) and that a single 3-h pulse did not induce DNA synthesis (SI = 1.2) in murine spleen cells. The discrepancy between this result and my own may be due partly to the presence of serum in the experiments of Toyoshima *et al.*, since their control cultures showed a high background of DNA synthesis presumably caused by the nonspecific mitogenic action of serum. In my cultures the absence of serum maintains the cells in a quiescent state but able to respond specifically to added mitogen, as indicated by the high stimulation indices of around 200.

Colchicine is known to bind tubulin and thereby reduce the microtubule system in cells. Its effect on lymphocyte mitogenesis in the continuous presence of con A has led to conflicting published results. For example, Betel and Martijnse[5] found that colchicine had no effect on the activation of rat lymphocytes by con A, but that the drug did block subsequent events in the cell cycle. In contrast, others[6] found that the inhibitory effect of colchicine was limited to the time of lymphocyte activation. In my own experiments using synchronously activated spleen cells, I found that short pulses of colchicine during the activation time caused a marked inhibition of mitogenesis (see Table 1). A 1-h pulse of colchicine immediately following con A treatment, however, had little effect on con A mitogenesis (Table 1). This supports the idea that colchicine inhibits events which are involved in the activation of cells. It was also found that the presence of colchicine during the final 24 h of a 52-h culture of cells (activated by a 2-h pulse of con A) caused marked inhibition of DNA synthesis measured at 47–52 h. These results suggest that an intact microtubular system is necessary for both mitogenic activation and the normal progression of the cell through the cell cycle.

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Development of mast cells from grafted bone marrow cells in irradiated mice

BOTH basophilic granulocytes and tissue mast cells are known to have receptors for immunoglobulin E (IgE) and granules which contain histamine and heparin[1,2]. In spite of their similar physiological role, these two types of cells are thought to be independent cell lineages; the former are derived from the bone marrow, and the latter are connective tissue cells[2]. The differentiation of mast cells from their precursors, which were supposed to be undifferentiated mesenchymal cells, and the proliferation of these precursors were reported to occur in the skin of mice[3,4]. These results do not necessarily exclude the possibility that the precursor cells themselves are originated from the bone marrow, however. To examine such a possibility, we investigated whether mast cells of the donor origin appeared in radiation chimaeras, using the giant granules of beige (Chediak–Higashi syndrome) mouse[5] as a quantitative marker for mast cells. We have found that tissue mast cells can be derived from grafted bone marrow cells in irradiated mice.

Beige (C57BL-Bg^J/Bg^J) mice (Jackson Laboratory, Bar Harbor, Maine) and normal (C57BL−+/+) mice were bred in

Table 1 Appearance of donor-type mast cells in various tissues

Mouse no.	Time after transplantation (d)	% of donor-type mast cells Caecum	Mesentery	Skin	Glandular stomach	Forestomach
M12	9	0	0	0	0	0
M25	24	0	0	0	0	0
M28	42	9	0	0	6	0
M04	63	96	0	0	79	1
M01	96	78	2	0	66	5
M10	126	79	6	0	57	26
M26	152	98	11	14	87	46
M54	180	94	2	0	56	36

The appearance of mast cells with giant granules was monitored at various times after the transplantation of bone marrow cells of the beige mice into the irradiated normal mice. The method of transplantation was described previously[12].

our laboratory. At an age of 3 months, normal mice were irradiated (800 rad) and injected intravenously with bone marrow cells (5×10^6) of either beige or normal mice. On various days after the cell injection, a blood sample was obtained from a lateral tail vein of recipient mice. The mice were then killed; the caecum, the stomach and a piece of dorsal skin were removed and fixed in 10% buffered formalin (*p*H 7.2). The stretch preparation of the mesentery was also fixed in 10% formalin. Blood smears were fixed in formaldehyde vapour, stained with Sudan black B, and counterstained with Giemsa solution[6,7]. More than 100 neutrophils with distinctly segmented nuclei were counted. Tissues were embedded in paraffin; sections and the streched mesentery were stained with acidified toluidine blue[5]. In most cases, more than 200 mast cells were counted in each tissue.

Over 90% of neutrophils had giant granules after the 8th day of the cell injection, and were considered to be of donor origin.

Examination of tissues under the microscope revealed the following. (1) Most of mast cells in the caecum were of donor origin after the 63rd day of the cell injection (Table 1). (2) Most of mast cells in the mesentery and the skin were of host type even on the 180th day (Table 1). (3) In the glandular stomach, mast cells of the donor type were predominant, whereas those of the host type were superior in the forestomach which is covered with stratified squamous epithelium (Table 1). (4) No mast cells with giant granules were found in any of control mice which had received the marrow cells of the normal mice after irradiation.

Our results clearly shown that most of the mast cells under glandular epithelium are originated from the grafted marrow cells and that mast cells of the host remained predominant under squamous epithelium and in the mesentery. As mast cells constitute less than 0.01% of the nucleated cells in the bone marrow of C57BL mice (both Bg^J/Bg^J and +/+, unpublished), and as it takes about 40 d for the mast cells of the donor origin to appear, the donor-type mast cells found in various tissues seem to be derived from the precursor cells rather than from the mature mast cells contained in the marrow of the donor.

Two possibilities are postulated to explain the difference in the proportion of the donor-type mast cells in the various tissues. (1) Mast cells under glandular epithelium may be different from those of other tissues. Enerbäck found that granules of some mast cells in the intestinal canal of the rat were not fixed with 10% formalin[8]. Moreover, intestinal mast cells stained according to the method of Enerbäck decreased in nude mice[9], although mast cells of the skin increased in these mice[10,11]. As 10% formalin was used for fixation in the present experiment, the mast cells counted in the intestinal canal should be different from the "mucosal mast cells" of Enerbäck. (2) Mast cells in the various tissues are of the same kind, but the turnover rate of the cells is different in each tissue presumably because the differentiation from the precursor cells is locally controlled. Further investigation is in progress to see whether the cellular contact or chemical factor, which act only in the short range, have a major role for the differentiation of mast cells.

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Evidence for Fc receptors on rabbit yolk sac endoderm

APPLICATION of the erythrocyte-antibody rosette technique (EA rosette) to cell cultures of mouse yolk sac membranes has provided evidence of Fc receptors on yolk sac endoderm of this species[1]. Binding of immunoglobulin (Ig) to Fc receptors forms an integral part of a hypothesis proposed by Brambell[2] to account for selective materno-foetal transmission of antibodies. In this hypothesis the receptor protects attached Ig when a transporting endocytotic vesicle (be it in syncytiotrophoblast, yolk sac endoderm, or gut enterocytes, according to the species) contacts proteolytic enzymes after fusion with lysosomes. Little is known about selective transport of antibodies across mouse yolk sac endoderm and it is not clear[1] to what extent these results show binding specific for transport of Ig across the endodermal cells, or merely reflect some special mechanism for endocytosing this particular class of proteins. Studies of rabbit yolk sac endoderm have not shown whether specific binding of Ig takes place; thus bovine IgG, which is poorly transported across rabbit yolk sac endoderm[3,4], although readily endocytosed[5], binds to the same extent as rabbit IgG to membrane components isolated from the cells[6]. On the other hand, compared with rabbit IgG, relatively little specific binding of bovine IgG was found when these proteins were incubated *in vitro* with formalin-fixed rabbit yolk sac[7]. We have now applied the EA rosette technique to rabbit yolk sac endoderm and find Fc receptors which bind both rabbit and human IgG, but not bovine IgG, pointing to their association with selective Ig transport.

To obtain yolk sac endodermal cells, conceptuses at 24–26 d of gestation were removed intact from pregnant Dutch or New Zealand White rabbits and washed free of contaminating blood with Hanks Balanced Salt Solution (BSS), *p*H 7.5. This was used free of calcium and magnesium to aid cell separation in subsequent procedures. Previous unpublished observations have shown that the exocoelomic fluid of the rabbit conceptus is rich in macrophages, as is the mesenchymal tissue of the paraplacental chorion[5]. In order to reduce possible contamination by macrophages and to provide a control population of Fc receptor positive cells, the exocoelomic fluid was removed by inserting a Pasteur pipette through a slit made in an avascular region of the chorion and flushing out the cavity with BSS. Flushing from different conceptuses were pooled. The yolk sac splanchnopleur was then dissected free of chorion, placed in a Petri dish with its endodermal surface uppermost and washed with BSS so as to remove blood issuing from the cut ends of vessels and macrophages possibly adhering to the exocoelomic mesothelium. BSS containing 0.5% trypsin and 10% foetal calf serum (FCS) was then added and incubation carried out at 37 °C for 1.5 h. The incubation medium was then carefully removed and fresh BSS/10% FCS pipetted on to the yolk sac endoderm so as to dislodge the cells. These were pooled from different conceptuses of the same rabbit and washed three times in a large volume of BSS/10% FCS. Between washings it was found necessary to pass cells through a hypodermic needle to achieve a single-cell suspension. Cells were finally suspended in Eagles Minimal Essential Medium containing 10% FCS and antibiotics at a concentration of 5×10^5 cells per ml.

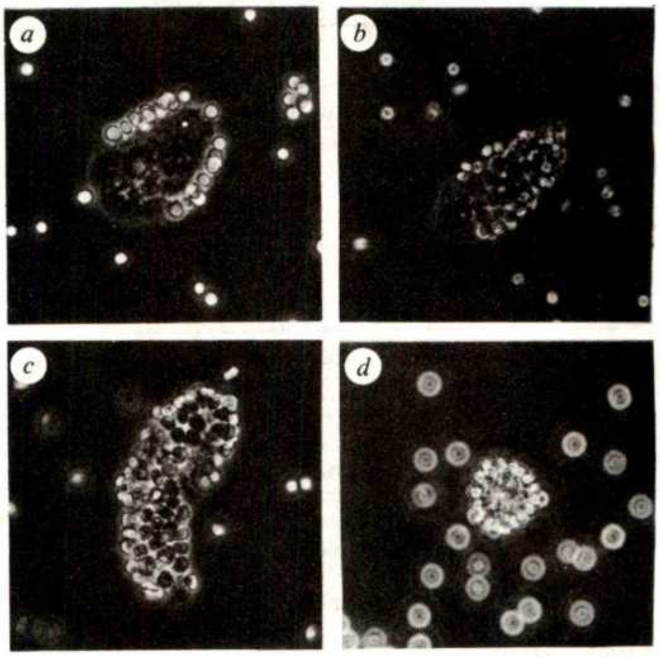

Fig. 1 Appearance of EA rosettes formed with yolk sac endodermal cells (*a*, *b* and *c*) and an exocoel macrophage (*d*). All photographs taken at the same initial magnification of ×298.

Table 1 Effect of rabbit antibody class and fragments on EA rosette formation with yolk sac endoderm (YSE) and exocoel macrophages (ExMac)

Sensitising antibody*	% Rosettes† obtained with YSE from animals 1–5					% Rosettes† obtained with ExMac from animals 1–4			
Whole serum	11	52	50	22.6	25	97	79	97	83
IgG	32.5	NT	NT	21.6	19	100	NT	97	NT
IgM	0	NT	NT	0	0	0	NT	7	NT
F(ab′)2	0	0	0	0	0	0	4	7	0
Fab	0	0	0	0	0	0	3.7	1.5	0
Control SRBC	0	0	0	0	0	0	3.5	4	0

NT, Not tested

*IgG, IgM, F(ab′)2 and Fab fractions derived from IgG were pure as defined by immunoelectrophoresis.

†Rosettes were formed by mixing equal volumes of YSE or ExMac and a 0.7% suspension of appropriately sensitised SRBC (tested for sensitisation with goat anti-rabbit immunoglobulins). Such mixtures were centrifuged at 150*g* for 3 min and the pellet gently resuspended. Aliquots were then mounted beneath coverslips on microscope slides and observed with ×40 phase objective. Cells showing at least five adherent SRBC were scored as positive. At least 100 cells were counted and the percentage of YSE or ExMac forming rosettes calculated. Duplicate samples normally showed agreement to within 5%.

A variable but significant number of rosettes were formed between yolk sac endodermal cells and sheep red blood cells (SRBC) coated with rabbit IgG; control SRBC and SRBC coated with IgM, and with F(ab′)2 or Fab fragments of IgG, did not form rosettes (Table 1). These results therefore show the presence of a receptor for the Fc region of rabbit IgG on at least a proportion of yolk sac endodermal cells; the variability observed is similar to that found for mouse yolk sac endoderm[1]. A very high percentage of exocoel macrophages also formed Fc dependent rosettes but we are confident these were not the source of rosetting cells in yolk sac endodermal cell preparations. By observing and counting rosettes under high power phase contrast microscopy, yolk sac endodermal cell rosettes could readily be distinguished from those formed by exocoel macrophages by their difference in morphology. Rosetting yolk sac endodermal cells frequently retained their columnar shape and sensitised SRBC were seen attached either in a band mainly at one pole (Fig. 1*a*) or distributed more widely over the cell surface (Fig. 1*b*, *c*). Exocoel macrophage rosettes differed by being smaller and very round in appearance with attached SRBC covering the cell surface (Fig. 1*d*). Several factors could have influenced the proportion and also the type of rosetting yolk sac endodermal cells observed. Although there may well be genuine differences between endodermal cells with respect to whether or not they possess Fc receptors, receptor sites might also have been blocked on some cells by endogenous IgG present within the uterine lumen; such IgG might be differentially released by the action of trypsin. Longer exposures of yolk sac splanchnopleur to trypsin-containing incubation medium did not have any consistent effect on the percentage of yolk sac endodermal rosettes subsequently scored, but it has been reported that treatment of formalin-fixed yolk sac splanchnopleur with trypsin reduces subsequent specific rabbit IgG binding by about 75% (ref. 8). Movement of receptors at some stage after isolation of cells from what is normally a polarised epithelium, might explain why attachment of sensitised SRBC occurs at sites other than what may be construed as the apical cell surface. But, no increase in the number of cells with SRBC attached more widely over the cell surface was seen when cell suspensions were further incubated after initial rosette counts. In an alternative mechanism to that proposed by Brambell, it has been suggested[9] that the receptor functions not so as to protect immunoglobulin from proteolysis within phagolysomes, but rather to segregate, at the cell surface, immunoglobulin destined for transport and that destined for proteolysis. It was envisaged that the receptor became associated with a distinct class of transporting coated micropinocytotic vesicles and subsequent studies[10] have detected rabbit and human IgG, but not bovine IgG, in coated vesicles in confluence with the lateral and basal plasmalemma. Thus endodermal cells engaged in transporting IgG may normally have free receptors generated at sites other than the apical cell surface as a result of coated vesicles fusing with the plasma membrane and these might account for the wide distribution of attached SRBC.

Rabbit, human and bovine IgG solutions were tested at two different protein concentrations for their ability to inhibit attachment of rabbit IgG sensitised SRBC to yolk sac endodermal cells. Rabbit and human Ig were most effective in this respect, but little or no inhibition occurred with bovine Ig (Table 2). Human IgG and rabbit IgG Fc were also very effective in inhibiting attachment but human IgM and rabbit IgG Fab had very much less, and in some cases, no effect. Rabbit albumin was also ineffective; indeed for some unknown reason this consistently enhanced the number of yolk sac endodermal cells forming rosettes with sensitised SRBC. These results thus confirm the importance of the Fc region of IgG for attachment to receptors on yolk sac endoderm. They also indicate that rabbit and human IgG bind to the same Fc receptor but that little if any binding of bovine IgG, human IgM or rabbit serum albumin occurs to this same receptor. The possibility does of course exist that separate receptors are present for these non-competing proteins, but that this is not the case for bovine IgG was shown by immunising a cow with SRBC and sensitising the SRBC with antibodies present in whole serum or the IgG fraction of this serum. As can be seen from Table 3, bovine IgG sensitised SRBC, unlike rabbit IgG sensitised SRBC, do not form rosettes with yolk sac endodermal cells. This could not be attributed to lack of a comparable degree of sensitisation, since exocoel macrophages showed a similar high level of rosette formation in parallel studies carried out with bovine or rabbit IgG sensitised SRBC. As mentioned above, bovine IgG is not transported across yolk sac endoderm to any significant extent, although it is readily endocytosed. Rabbit and human IgG are more readily transported across rabbit yolk sac endoderm and all three immunoglobulins can be detected in phagolysosomes[11]. Since there is no apparent binding of bovine IgG to an Fc receptor, the inescapable conclusion seems to be that there is an association between the Fc receptor and selective immunoglobulin transport. Clearly, yolk sac endodermal cells, in view of their difference in binding affinity for bovine IgG, must have subtle differences in the nature of the Fc receptor compared with that present on macrophages. The presence of Fc receptors specific for IgG has also been demonstrated on human syncytiotrophoblast, both by EA rosette formation[12] and immune cytoadherence[13]. Also, binding of IgG to the human placental receptor seems to be dependent on an intact Fc region since neither C2 or C3 homology regions would competitively inhibit binding of IgG (ref. 14). This is again different to the macrophage Fc receptor, which will bind the isolated C 3 homology region[15].

Table 2 Inhibition of YSE–EA rosette formation by homologous and heterologous proteins

Protein tested* (mg ml^{-1})		Mean % inhibition†	Range	No. of animals
Rabbit Ig				
0.5		78.5	68.0–83.3	4
5.0		95.0	90.0–100	3
Human Ig				
0.5		93.4	91.1–95.4	4
5.0		100	100 –100	3
Bovine Ig				
0.5		14	0.0–28.0	4
5.0		(–)3.0	(–)14.3–17.1	3
Human IgG		85.9	83.1–88.7	2
Human IgM	all at	6.8	0.0–13.6	2
Rabbit IgG Fc	0.5 mg ml^{-1}	71.4	60.2–78.5	3
Rabbit IgG Fab	final conc.	2.0	(–)35.5–30.0	3
Rabbit albumin		(–)6.2	(–)2.2–(–)11.5	3

YSE cells were mixed with equal volumes of protein solution and left at room temperature for 30 min before adding equal volumes of either sensitised or control SRBC. Rosettes were then enumerated as described in Table 1. All tests carried out with control SRBC were negative in these experiments.

*Human and bovine Ig were obtained from Koch-Light (Cohn Fr. 2) and rabbit Ig from Calbiochem; they were principally IgG.

† % Inhibition = 100 – (% rosettes formed between IgG sensitised SRBC and YSE in presence of competing protein/% rosettes formed in absence of competing protein × 100).

Table 3 Effect of rabbit and bovine IgG sensitising antibody on EA rosette formation with yolk sac endoderm (YSE) and exocoel macrophages (ExMac)

Source of sensitising antibody to SRBC	Mean % rosettes* for YSE and range	Mean % rosettes* for ExMac and range	No. of animals
Rabbit whole serum	11.8 (8.0–19.0)	97.0 (94.0–100)	4
Rabbit IgG fraction	26.3 (16.0–45.5)	81.6 (52.0–96.5)	6
Bovine whole serum	0 (0)	56.2 (25.0–100)	4
Bovine IgG fraction	0.2 (0–1.0)	75.8 (50.0–96.0)	6
Control SRBC	0.2 (0–2.0)	3.5 (0–11.0)	10

*Determined as described in Table 1.

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Localisation of gonadotropin-releasing hormone in the circumventricular organs of human brain

Gonadotropin-releasing hormone (GnRH) and thyrotropin-releasing hormone (TRH) are two hypothalamic oligopeptides which regulate the secretion of gonadotropins and thyrotropin, respectively, from the anterior pituitary[1,2]. It is generally accepted that these neurohormones are produced by nerve cells, transmitted along axons and stored in nerve endings. When released, they are transferred by way of the hypothalamo–hypophyseal portal system to the anterior pituitary[3]. We have recently reported[4] that in the human hypothalamus most of the GnRH is found in the preoptic area while TRH is localised mainly in the posterior nucleus. Exceedingly high concentrations of the two neurohormones were found in the pituitary stalk. The localisation of the two neurohormones in distinctive regions of the hypothalamus and pituitary stalk may represent sites of synthesis in neuronal cell bodies and storage in nerve terminals, respectively. It has recently been found[5] that significant amounts of GnRH are present in the circumventricular organs (CVO) of the rat brain. These organs consist of specialised ependymal cells, small nerve cells and axon terminals containing small dense core vesicles. The axon terminals end on fenestrated capillaries comprising many capillary loops[6]. Such an anatomical arrangement is characteristic of neurohumoral secretion, and is present also in the human pituitary stalk[7]. In this study we have determined, by radioimmunoassay, the distribution of GnRH and of TRH in the circumventricular organs of the human brain. We report that exceedingly high concentrations of GnRH, but not of TRH, are present in one of the circumventricular organs, the organum vasculosum of the lamina terminalis (OVLT), and discuss the possible physiological function of this structure in the neural control of reproduction.

Different brain structures were removed during routine autopsies carried out at the Institute of Forensic Medicine, Tel Aviv. The tissues were obtained from six females, 19–70 yr old, and from four 30–70-yr-old males, seven of them killed in road accidents and three suicide cases. The bodies were kept for 4–52 h at 4 °C until autopsy. Identical storage conditions did not affect GnRH or TRH content of brain tissue[4]. The circumventricular organs (Fig. 1) and the tissues surrounding them were removed under a dissecting microscope and sectioned into several blocks. The pituitary stalk and specific hypothalamic regions were sectioned as described previously[4]. Each of the segments (52 tissue blocks per brain) was extracted and analysed for GnRH and TRH by radioimmunoassay, as described previously[8,9]. Protein determination was performed according to the Lowry method[10]. The pineal body, a circumventricular organ, was excluded from this study since we have previously shown that it does not contain any significant amount of GnRH or TRH (ref. 4).

The largest amount of GnRH and TRH was found in the pituitary stalk (52ng of GnRH and 21 ng of TRH). A considerable amount, however, of GnRH (9.4 ng) was also found in the OVLT (Table 1); its concentration in this structure (36.4 ng per mg protein), exceeded even that found in the pituitary stalk (20.7 ng per mg protein). The lamina terminalis surrounding the vascular structure, as well as the other CVO, contained negligible amounts of GnRH. Relatively low concentrations of TRH were found in all the CVO (Table 1). Within this limited series, neither the amount and concentration of GnRH in the OVLT and pituitary stalk, nor the distribution of the neurohormone between these two organs could be shown to be related to age or sex of the subjects.

It seems likely that in the human, the OVLT, like the pituitary stalk, functions as a neurosecretory organ: the specific localisation and high concentration of GnRH in both organs, and the presence of a rich vasculature, and of nerve endings terminating on capillary loops, would suggest neurohumoral secretion of the hormone at these two sites. The physiological significance of GnRH secretion at the pituitary stalk is well established: the hormone is carried by way of the hypothalamo–hypophyseal portal system to the anterior pituitary, thereby inducing gonadotropin release. The role of GnRH originating in the OVLT is not clear. Transport of the hormone to the anterior pituitary by the circuitous route of the cerebrospinal fluid or by the general circulation seems unlikely. Alternatively, the drainage of the OVLT towards the preoptic area in the human[11] could provide a pathway for distribution of GnRH to diverse brain regions. In these areas, the hormone could modulate neurotransmittory functions or influence the activity of different neurones.

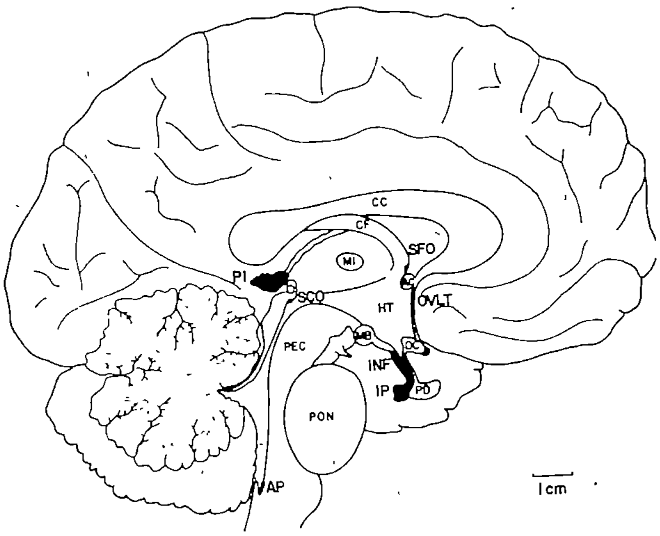

Fig. 1 The circumventricular organs of the human brain (in black), in a midsagittal plane section. AP, area postrema; INF, infundibulum of neurohypophysis (pituitary stalk); IP, infundibular process of neurohypophysis (posterior pituitary); OVLT, organum vasculosum lamina terminalis; PI, pineal body; SCO subcommissural organ; SFO, subfornical organ. Other structures: AC, anterior commissure; CC, corpus callosum; CF, column of fornix; Ht, hypothalamus (shaded area); MB, mammillary body; MI, massa intermedia; OC, optic chiasma; PD, pars distalis of adenohypophysis; PEC, peduncle of cerebrum; PON, pons.

Several observations suggest a possible relationship between the OVLT and behavioural and endocrine parameters of sexual function. Destruction of the preoptic area, to which the OVLT is adjacent, modifies sexual behaviour in several animal species[12] and disrupts the female oestrous cycle of the rat[13]. Wenger[14] has recently shown a correlation between the endocrine state of the female rat and the number of dense core vesicles in the OVLT resembling those of the GnRH-containing vesicles of the median eminence of the hypothalamus. Moreover, a direct effect of GnRH on sexual behaviour has been demonstrated by Moss and McCann[15] and by Pfaff[16], who by administration of GnRH to ovariectomised or ovariectomised hypophysectomised rats were able to induce mating behaviour.

While in the rat brain considerable concentrations of GnRH were found in all the CVO (ref. 5), the human brain

Table 1 GnRH and TRH in the CVO of the human brain

Structure	GnRH		TRH	
	Content (ng)	Concentration (ng per mg protein)	Content (ng)	Concentration (ng per mg protein)
Pituitary stalk	51.98±9.69*	20.75±1.62⁺	21.30±3.38	13.71±2.24†
Vascular organ of lamina terminalis	9.42±1.67	36.40±5.22	0.34±0.18	1.31±0.69
Subfornical organ	0.85±0.26	1.21±0.37	0.64±0.18	0.91±0.25
Subcommissural organ	0.35±0.20	0.50±0.28	0.26±0.14	0.37±0.20
Area postrema	0.13±0.11	0.18±0.15	0.74±0.13	1.05±0.18
Preoptic area of hypothalamus	4.78±0.67‡	1.86±0.51	3.92±0.31‡	1.53±0.48
Cerebral cortex		<0.03		0.06±0.04

*Mean ± s.e.m. for 10 samples are shown.
†Peak concentrations of GnRH and TRH in the upper portion of the pituitary stalk.
‡Content of the POA of half a hypothalamus, dissected in the midsagittal plane.

contains high concentrations of GnRH in the pituitary stalk and OVLT only. The basic anatomical resemblance of the OVLT and the pituitary stalk suggests that GnRH is secreted from the vascular organ as well. It is possible that the GnRH secreted from the OVLT may have a role in the regulation of the menstrual cycle and sexual behaviour. However, further work is required to establish the exact physiological role of GnRH localised in the OVLT.

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Chemical carcinogens stimulate canine kidney (MDCK) cells to produce prostaglandins

WHILE investigating the regulation of prostaglandin production by cells, I found that the MDCK line from dog kidney[1] responds to treatment with several polycyclic aromatic hydrocarbon carcinogens, aflatoxin B_1 and 1,2,7,8-diepoxyoctane. Metabolic activation of the hydrocarbons was required for this stimulation of prostaglandin production.

The MDCK cell line, which morphologically resembles epithelium, was obtained from Dr Charles D. Stiles, University of California, San Diego. Exponentially growing cells were incubated in Eagle's minimal essential medium containing 2 mM *l*-glutamate and supplemented with 10% (v/v) foetal bovine serum and penicillin–streptomycin. Duplicate dishes were used for each reagent and each experiment was repeated at least once. The values obtained for prostaglandins per cell were similar for each reagent in all experiments. Media were assayed for PGE_2 and $PGF_{2\alpha}$ with either anti-PGE_2 or anti-$PGF_{2\alpha}$: anti-$PGF_{2\alpha}$ reacts 0.1% with PGE_2 and the anti-PGE_2 reacts 0.01% with $PGF_{2\alpha}$. (The antisera do not identify the prostaglandins as either monoenoic or dienoic.)

MDCK cells produce prostaglandins—about eight times more $PGF_{2\alpha}$ than PGE_2. When the cells were incubated in the presence of benzo(*a*)pyrene (0.1 μg ml^{-1}), the content of $PGF_{2\alpha}$ and PGE_2 in the medium increased. In my experiments the production of prostaglandins by MDCK cells and its stimulation in the presence of benzo(*a*)pyrene depended on cell density. At all densities, however, the cells produced greater levels of prostaglandins in the presence of benzo(*a*)pyrene (0.1 μg ml^{-1}).

When cells were plated at 2×10^5 per dish, stimulation by benzo(*a*)pyrene (0.1 μg ml^{-1}) was observed after 48 h of incubation. Benzo(*a*)pyrene was not required throughout the 48 h. There was equivalent stimulation if cells were incubated at 2×10^5 per dish for 24 h with benzo(*a*)pyrene, washed twice and incubated for a further 24 h in medium lacking benzo(*a*)pyrene. This procedure therefore was used to test the various reagents. Medium from the second 24-h incubation was assayed for serological activity with anti-$PGF_{2\alpha}$ and anti-PGE_2.

Table 1 shows that the cells incubated with the most active polycyclic aromatic carcinogens[2-6] produced the highest levels of prostaglandins. Incubation with benzo(*a*)pyrene at 0.5 μg ml^{-1} stimulated prostaglandin production 13-fold and at 0.2 μg ml^{-1}, 5–5.5-fold. Among the fungal toxins tested, only the most potent carcinogen, aflatoxin B_1 (ref. 5), at 0.05 and 0.02 μg ml^{-1}, stimulated prostaglandin production, while the weakly active carcinogenic aflatoxins, at 0.1 μg ml^{-1}, did not. Neither the aromatic amines nor the nitrosamines, at 0.5 μg ml^{-1}, affected prostaglandin production.

The direct-acting carcinogen, 1,2,7,8-diepoxyoctane, at 0.5 μg ml^{-1}, stimulated prostaglandin production 3–3.5-fold. The 1.7-fold stimulation due to chrysene (0.5 μg ml^{-1}) was statistically significant (0.533 ± 0.05 fg of PGE_2 per cell (medium) and 0.903 ± 0.19 fg per cell (chrysene) $P < 0.005$; and 2.92 ± 0.26 fg $PGF_{2\alpha}$ per cell (medium) and 4.93 ± 0.48 fg per cell (chrysene) $P < 0.005$).

Table 1 Prostaglandin production by MDCK cells

Effector*	Increase in prostaglandin production		Carcino-genicity
	PGE_2†	$PGF_{2\alpha}$†	
Medium (control)	1.0	1.0	—
Benzo(*a*)pyrene	13.6	12.2	+
Benzo(*a*)pyrene§	5.1	5.7	+
7,12-Dimethylbenz(*a*)anthracene	10.9	10.6	+
3-Methylcholanthrene	4.1	4.8	+
7-Methylbenzanthracene	4.2	3.9	+
5-Methylchrysene	3.6	3.8	+
3-Methylchrysene	2.0	2.1	+
Chrysene	1.7	1.7	+
1-Methylchrysene	1.4	1.6	—
Dibenz(*a*,*h*)anthracene	1.0	1.4	+
Benz(*g*,*h*,*i*)perylene	1.2	1.1	+
Benz(*a*)anthracene	1.2	1.5	+
1,2,3,4-Dibenzanthracene	1.0	1.0	—
Anthracene	0.8	0.9	—
Phenanthrene	0.7	0.3	—
Pyrene	0.8	1.1	—
Perylene	0.8	1.2	—
Aflatoxin B_1¶	4.6	5.0	+
Aflatoxin B_1‡	2.6	2.2	+
Aflatoxin B_2**	0.7	1.0	+
Aflatoxin G_1**	1.0	1.2	+
Aflatoxin G_2**	1.2	1.3	+
2-Aminoanthracene	1.3	1.3	+
2-Aminofluorene	0.9	1.0	+
6-Aminochrysene	1.7	1.5	+
2-Acetoaminofluorene	1.5	1.3	+
4-Aminobiphenyl	1.1	1.2	+
Dimethylnitrosamine	1.2	1.1	+
Diethylnitrosamine	1.1	1.2	+
Dibutylnitrosamine	1.5	1.6	+
1,2,7,8-Diepoxyoctane	3.5	3.0	+
5,6-Benzoflavone**	1.1	1.2	—
7,8-Benzoflavone**	1.2	1.3	—
SKF 525-A††	1.2	1.0	—
Metyrapone††	1.3	1.0	—
1,2-Epoxy 3,3,3-trichloropropane††	1.2	1.1	—
Nicotine	1.0	1.1	—
Phenobarbital‡‡	0.9	1.0	—
Dimethylsulphoxide§§	1.0	1.1	—

*Unless specifically noted, 0.5 μg ml^{-1} was used.

†Expressed as relative to level measured after incubation in medium alone (1.0); for example, a value of 13.6 represents a 13.6-fold increase in prostaglandin production. Carcinogenic activities obtained from the literature[2-6].

§0.2 μg ml^{-1}.

¶0.05 μg ml^{-1}.

‡0.02 μg ml^{-1}.

**0.1 μg ml^{-1}.

††1.0 μg ml^{-1}.

‡‡10.0 μg ml^{-1}.

§§0.05%.

Several polycyclic aromatic hydrocarbons stimulated prostaglandin production. Metabolism of these compounds to arene oxide products by enzymes such as aryl hydrocarbon hydroxylases are required for carcinogenic activity[6,7]. Such metabolism can be blocked by 7,8-benzoflavone[8,9]. 7,8-Benzoflavone inhibited stimulation by benzo(*a*)pyrene, 7,12-dimethylbenz(*a*)anthracene and aflatoxin B_1, but not that of 1,2,7,8-diepoxyoctane; 7,8-benzoflavone, on the other hand, increased stimulation by the latter (Table 2). The inhibitors of microsomal monoxygenases, SKF 525-A and metyrapone[10] (0.1 μg ml^{-1}) inhibited stimulation by both benzo(*a*)pyrene and 1,2,7,8-diepoxyoctane by 20%; while 1,2-epoxy 3,3,3-trichloropropane (1.0 μg ml^{-1}), an inhibitor of epoxide hydrase, had no effect.

Table 2 Inhibition by 7,8-benzoflavone of stimulation of prostaglandin production in MDCK cells by benzo(*a*)pyrene

Effector	μg ml^{-1}	$PGF_{2\alpha}$ (fg per cell)	Increase in prostaglandin production*
No addition	—	3.9	1.0
7,8-Benzoflavone	0.1	3.6	0.9
7,8-Benzoflavone	0.01	3.7	0.9
7,8-Benzoflavone	0.001	3.8	1.0
Benzo(*a*)pyrene	0.1	16.7	4.3
+7,8-Benzoflavone	0.1	4.1	1.1
+7,8-Benzoflavone	0.01	9.4	2.4
+7,8-Benzoflavone	0.001	16.8	4.3
7,12-Dimethylbenz(*a*)anthracene	0.5	40.1	10.3
+7,8-Benzoflavone	0.1	9.0	2.3
Aflatoxin B_1	0.05	16.4	4.2
+7,8-Benzoflavone	0.1	6.2	1.6
1,2,7,8-Diepoxyoctane	0.5	11.7	3.0
+7,8-Benzoflavone	0.1	19.9	5.1

*Expressed as relative to level found in medium alone (1.0); for example, a value of 4.3 represents a 4.3-fold increase in prostaglandin production.

After incubation with the carcinogens that stimulated prostaglandin production, trypsin-treated MDCK cells were larger than either trypsin-treated control cell or cells incubated with non-stimulating reagents. No attempt, however, was made quantitatively to correlate this increase in cell size with increased prostaglandin production.

Greater prostaglandin production by transformed cell lines than by their parent cell lines has been reported[12,13]. Methylcholanthrene-transformed mouse fibroblasts produce more prostaglandins because their synthetase activity is higher[13]. Stimulation of the activity of the microsomal enzyme complex prostaglandin synthetase could also account for the stimulation reported here. On the other hand, increased synthetase activity may reflect the morphological changes in the cells, with no induction of enzyme. Increased phospholipase activity, either as a result of induction of phospholipases or as a consequence of the morphological alterations, could also account for the stimulated prostaglandin production by increasing the availability of arachidonic acid, the substrate for prostaglandin synthetase.

Human fibroblasts (D550), rabbit endothelial cells (CLO) and mouse fibroblasts (3T3), although they produce prostaglandins, were unaffected by benzo(*a*)pyrene (0.2 μg ml^{-1}; 48 h incubation). MDCK cells probably contain the metabolic enzymes necessary to convert the procarcinogens to prostaglandin-stimulating compounds, but they do not seem to have those required to activate aromatic amines or nitrosamines to stimulators of prostaglandin production; other cell lines which may have these enzymes are being studied. The relationship of carcinogenicity to stimulation of prostaglandin production at the concentrations used is positive (Table 1). With increased concentrations of some of the reagents (5 μg ml^{-1} and above), the false negatives may be positive. But toxicity becomes marked at higher levels and a procedure to quantify the contributions of the toxic and non-toxic effects to prostaglandin production is being developed. The MDCK cell line may prove to be a sensitive indicator of the carcinogenic potential of the hydrocarbons.

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Infectious primate type-C virus shed by healthy gibbons

MANY captive gibbons are naturally exposed to infectious type-C virus based on the prevalence of animals with humoral antibodies reactive to the virus[1,2] and isolation of virus from tissue of leukaemic[3,4] and normal gibbons[5]. A closely related infectious type-C virus has also been isolated from a woolly monkey with a spontaneous fibrosarcoma[6,7] which implies that a common virus could infect unrelated non-human primates. Several reports recently have identified infectious primate type-C virus[8,9], viral components[10] and humoral antibody reactive to this virus in leukaemic[11] and normal humans[12] suggesting that man also may be susceptible to infection by a related virus. In an attempt to identify a possible source for infectious primate type-C virus that might infect primates as well as other animals, we decided to investigate whether healthy captive gibbons were shedding viruss ince this non-human primate is frequently displayed at zoos or kept as pets. This report presents evidence that gibbons can have long term viraemia with type-C virus without detectable immune response. These viraemic animals actively shed infectious virus in urine and faeces.

Blood samples were taken monthly or when available from laboratory, pet and zoo gibbons (*Hylobates* sp.) for clinical, virological and serological studies. All gibbons under observation were initially free of any clinical symptoms. Blood samples (3–5 ml) were taken from the femoral vein for plasma and sera. Plasma was used to infect virus-susceptible cell cultures. Serum samples were used to detect the presence of humoral antibody.

Faecal and urine specimens were collected during the monthly physical examination. Each urine specimen was filtered through a 0.45 μm Millipore filter then concentrated by differential centrifugation. The sterile concentrate was resuspended in physiological buffered saline (PBS) and used as an inoculum. Each faecal sample was initially diluted as a 20% suspension in PBS. The suspension was subjected to low speed centrifugation to remove the large particles. The clarified suspension was filtered through a 0.45 μm Millipore filter, then subjected to high speed centrifugation to pellet the virus. The pellet was resuspended in PBS and used for inoculum.

Fifty-three gibbons were examined over 12 months for the presence of infectious type-C virus and viral-specific humoral antibody. All gibbons were separated into two major groups depending on whether the animals were or were not exposed to the virus. Animals in group 1 were considered normal, that is, free of detectable infectious virus and humoral antibody. Animals in group 2 were those animals which had been exposed to type-C virus as determined by either the presence of infectious virus or humoral antibody. Results are summarised on Table 1. Thirty-three of the 53 gibbons (62%) examined were considered normal. Two hundred and ten plasma samples from these animals lacked evidence for the presence of infectious virus and humoral antibody. None of these animals seroconverted during the study period.

Animals in group 2 were divided into two subgroups based on whether the animal had detectable humoral antibody or infectious viraemia. Two hundred and twenty-two blood samples from 14 antibody positive gibbons were examined for humoral antibody and virus. All the samples from subgroup A contained humoral antibody but none of the samples contained detectable infectious virus. The humoral antibody titre for viral envelope antigen in these animals generally ranged from 1/4 to 1/16 by IFA except for one animal which ranged from 1/32 to 1/128. The radioimmune precipitation assay for P_{30} ranged from 1/50 to 1/490 (ref. 14). Many of these animals were examined on several later occasions and in no instance did any of them convert from antibody positive to detectable viraemia. Tests on 38 samples from six viraemic gibbons (subgroup B), however, showed that all the samples contained infectious type-C virus but lacked detectable humoral antibody. One viraemic gibbon has been observed for over 4 yr and some have been observed for at least 1 yr. Four of the six gibbons are still under observation, two died after 6 and 12 month observations. One animal developed maligant leukaemia–lymphoma while the other died of unknown cause. All the remaining gibbons continue to be in good health.

Table 1 Gibbon antibody and virus detection

Group	Animals	Antibody	Virus
1 Normal	33	0/210	0/210
2 Virus-infected			
A Antibody-positive	14	220/220	0/210
B Antibody-negative	6	0/38	38/38

Detection of virus was performed by infecting the susceptible human lymphoid cell culture (NC-37) with the prepared inoculum. Approximately 2×10^6 viable cells were concentrated into a pellet and resuspended in 0.2 ml of inoculum. The suspension was agitated for 90 min and then fresh medium was added to each tube. After three passages (1:3) the virus was concentrated from the tissue culture supernatant and assayed for viral DNA-polymerase in the presence of oligo dT·poly rA[3]. Viable cells were examined for viral envelope antigen by direct immunofluorescence (IFA) as previously described[4]. All positive cell cultures were confirmed for replicating type C virions by electron microscopy[3].

Antibody reactive to type-C virus in gibbon sera was determined by indirect immunofluorescence assay (IFA) using viable infected and non-infected gibbon and human lymphoid cells as targets. The secondary antibody was FITC conjugated goat antihuman IgG or anti-monkey IgG as previously described[1].

Isolation of virus from viraemic gibbons was consistent which suggested that these animals probably had a high virus titre. In order to determine the approximate titre in the plasma, infectious virus was titrated by the endpoint dilution method. Results in Table 2 indicate that most gibbons had high virus titre. One animal (HLA-21), which was examined many times, showed a constant titre between 10^1 and 10^2 infectious virus (IV) ml^{-1}. These results indicate that viraemic gibbons can tolerate a very high virus titre without any overt ill effects.

Since infectious type-C virus could be consistently isolated from the blood of these viraemic gibbons, we examined urine and faecal samples from these animals to determine if infectious virus was being excreted which could serve as a possible source for dissemination of the agent. Results are summarised in Table 2. Infectious virus could be readily isolated from fresh urine and intermittently from faecal samples of viraemic gibbons. Isolation of infectious virus from excretory products seems to depend on the virus titre of the viraemic animals. None of the normal nor antibody-positive gibbons was found to excrete detectable levels of infectious virus in their urine or faeces.

The present study clearly indicates that healthy gibbons can have persistent type-C virus infection and can shed infectious virus. Gibbons must be highly susceptible to type-C infection in order to account for the prevalence of the virus found in this species. In most instances, the animal developed an active immunity probably resulting from low grade chronic multiplication of the virus in the tissues. A few animals with high level virus multiplication developed long term viraemia without detectable humoral antibody response, possibly because of partial immunological tolerance for the virus or because the virus multiplication exceeded the antibody response in the host. This situation may be similar to cats infected with feline leukaemia virus (FeLV) where the infected cats lack diseases or detectable immune response[15].

Table 2 Comparison of viraemia and detection of virus in urine and faeces

Animal no.	Age (yr)	Health	Virus	Detection of infectious virus	
			IV ml^{-1}	Urine	Faeces
HLA-21	6	normal	10^{1-2}	1/6	0/3
HLA-30	6	malignant leukaemia–lymphoma	10^4	ND	ND
HLA-68	3.5	normal	10^5	10/14	4/14
HLA-71	9	normal	10^5	14/16	10/16
HLA-72	2	normal	10^5	2/5	4/5
4 AB pos	4–6	normal	0	0/64	0/70

The gibbon virus is essentially identical to the woolly monkey fibrosarcoma virus[16,17] which has been demonstrated to be oncogenic in marmosets[18] and has characteristics in common with other known animal oncornaviruses. Based on molecular hybridisation analysis the infectious primate virus is exogenous to the species[19,20] and probably transmitted horizontally. The mode of transmission may be similar to FeLV[21]. While several investigators have reported detection or isolation of infectious primate type-C virus[8,9] and humoral antibody reactive to the agent in normal and leukaemic humans[11,12], other investigators were unable to confirm these findings[21]. This controversial point requires further study and needs to be resolved. Although there is no evidence that the gibbon agent is infectious to man, it is known that many non-human primate agents can infect man[23]. More importantly, this study indicates that there are gibbons which seem to be in good health in zoo and pet environment which are shedding infectious type-C virus and may be a source for dissemination of this virus. Four gibbons in this study were from such an environment and were essentially normal in their behaviour as far as appetite and activity. Routine clinical and physical examination of these animals showed no discernible abnormalities and only with specific immunological tests was the infection detected. One gibbon developed malignant leukaemia–lymphoma during this study which suggests the oncogenicity of this agent. The other viraemic gibbons may develop similar disease, however, at present they continue to be healthy and active. Further studies are needed to determine whether the gibbon agent is infectious and oncogenic to other primates.

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Spatial summation and light adaptation in the goldfish visual system

IN attempting to explain how visual acuity improves as illumination increases, Pirenne and Denton[1] suggested that the retinal image is sampled by numerous units, each summing the effects of light over a region of the retina. At very low luminances, only units with the largest summation areas are functional, but with increasing luminance smaller units come into play as their thresholds are exceeded. Since that suggestion was made, cells at all levels of the visual system have been studied electrophysiologically and many receptive fields have been described that might form the basis for such units. Goldfish and other cyprinid fishes, have been the subjects of many such studies, and their retinal synaptic connections have been revealed in detail. I report here some psychophysical experiments which show the effects of light adaptation on spatial summation in the goldfish. The results are consistent with the connections made by rods and cones in the cyprinid retina, and with the theory of a progressive recruitment of ganglion cells with smaller receptive field centres as background luminance is raised.

Radiance thresholds of goldfish (12 cm standard length) were measured by classical conditioning of respiration[2]. Each fish was restrained with its body parallel to a diffusing screen at 14 cm from the left eye. The test stimuli were disks of monochromatic light (533 nm) projected for 5 s in the centre of the fish's visual field. The animals were trained by giving a brief electric shock at the offset of the test stimulus so that the shock-elicited inhibition of breathing came to be evoked by the light stimulus. The occurrence of a response was judged by a 4-alternative forced choice procedure: the test stimulus was presented randomly in one of four 5-s periods within each minute. A response was counted if the stimulus period contained an interbreath interval longer than those in the other three periods. Thresholds were estimated from a staircase tracking procedure[3].

Figure 1 shows how threshold radiance changes with stimulus diameter when dark adapted (*a*), and against an adapting tungsten-light background of 3.4 mL (*b*). When light flux is summed perfectly, sensitivity (1/threshold) increases in proportion to stimulus area (Ricco's Law) up to the limit of full summation, and thereafter increases no further. Since the data can be fitted by two straight line segments, one of slope 2, representing Ricco's Law, and one of zero slope, the limit of full summation can be conveniently expressed by the 'critical diameter' (CD) at which the two lines intersect.

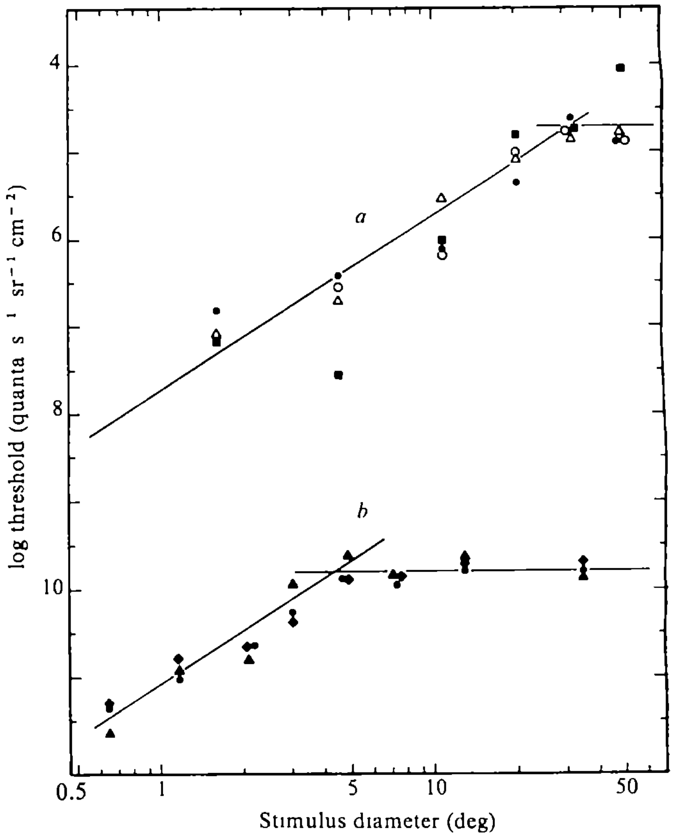


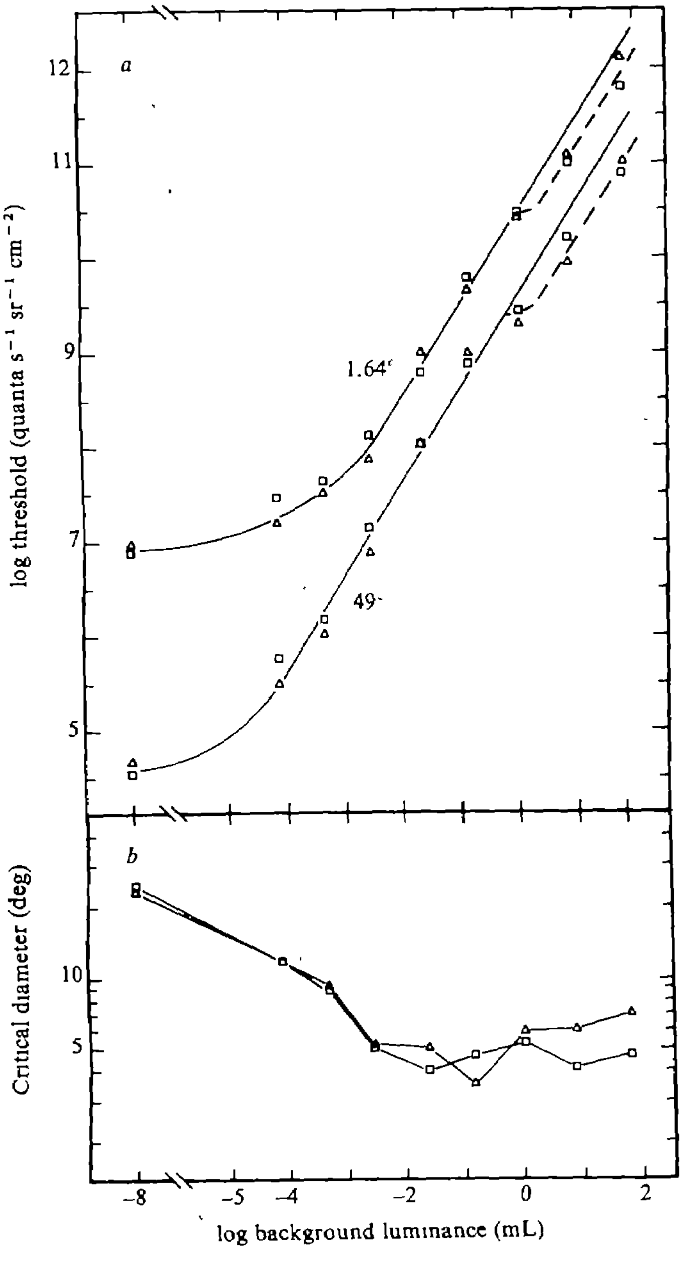
Fig. 1 Threshold stimulus radiance of wavelength 533 nm as a function of stimulus diameter at two different adaptation levels. Each symbol represents data from a different fish. The sloping lines show a reciprocal relationship between threshold and stimulus area (Ricco's Law). Lines fitted to data by eye. *a*, Thresholds obtained after at least 30 min of dark adaptation; *b*, thresholds to test stimuli projected on a tungsten-light background (3,000 K) of 3.4 mL.

In the dark-adapted state, full summation takes place for stimuli subtending up to about 30° of visual angle. With a 49° diameter stimulus, the mean absolute threshold of four goldfish in terms of stimulus radiance was 58,200 quanta s^{-1} sr^{-1} cm^{-2}, which is 38% of the human absolute threshold measured in similar conditions[4]. Using targets of more than 100°, goldfish thresholds fell to about 15% of the human value, indicating some partial summation above 30°. Very similar absolute thresholds to large stimuli have previously been obtained for goldfish[5].

To examine how sensitivity and spatial summation vary with the state of adaptation, thresholds in two goldfish to a small (1.64°) and large (49°) stimulus superimposed on different luminances of tungsten-light background were measured. Figure 2*a* shows that backgrounds have the usual desensitising effects: threshold rises at an increasing rate until it changes in proportion to background luminance (Weber's Law) as shown by the straight lines. At a background luminance of ~1 mL however, this linear relationship is interrupted by what seems to be a rod-cone break. A previous psychophysical study on goldfish found a similar break at about the same adaptation level[6].

Given that straight lines of slope 2 and 0 describe the threshold-diameter data (Fig. 1), CD can be estimated from the intersection of two such lines drawn through the 1.64° and 49° stimuli respectively. Thus CDs at different background luminances (Fig. 2*b*) were derived from the corresponding pairs of thresholds in Fig. 2*a*. CD in these two goldfish fell from a dark-adapted value of 25° to about 5° in a fairly continuous fashion, remaining essentially constant above 3×10^{-3} mL. In humans, however, CD falls with increasing background luminance in two distinct phases: first to an intermediate level while the rods determine threshold, and then to a lower level when the cones take over[7]. The fact that the goldfish CD falls without interruption, in spite of the break in the threshold–background luminance function, suggests that rod and cone signals are pooled over similar retinal areas. An equality of spatial summation in the rod and cone systems of goldfish, and the lack of it in humans can possibly be explained by the retinal organisation of the two species. In mammals, the bipolar cells contacted by rods are different from those contacted by cones[8], but in cyprinid fishes, several types of bipolar cell are contacted by both rods and cones[9,10]. Furthermore, these fish bipolar cells receive their rod and cone input from the same retinal area, presumably leading to equal rod and cone summation areas at the bipolar and subsequent levels of processing.

Fig. 2 *a*, Threshold stimulus radiance as a function of background luminance for 533-nm test disks of 1.64° and 49° diameter. △, And □, are data points from two goldfish of 12 cm standard length. The straight lines show Weber's Law and the dashed curves represent probable cone thresholds; *b*, relationship between critical diameter and background luminance. Each critical diameter (CD) in degrees of visual angle is found from the corresponding pair of thresholds (I_{49} and $I_{1.64}$) in *a* from $\mathrm{CD} = 1.64(I_{49}/I_{1.64})^{1/2}$.



It is unlikely that the spatial summation seen behaviourally in the goldfish could take place much before the retinal ganglion cells. As yet there is little physiological evidence for inter-receptor coupling in fishes as a basis for summation[11], although contacts between receptors have been found[10,12]. Anatomical and electrophysiological studies in cyprinid fishes show that the receptive fields of bipolar cells are smaller than the smallest behavioural critical diameters, being 2.5° in diameter at most[9,10,13], whereas the receptive fields of the laterally spreading elements, the horizontal and amacrine cells, are too large to account for the relatively small, light-adapted critical diameter[14,15]. Receptive field centres of ganglion cells are, however, found in a range of sizes that matches quite closely the range of critical diameters encountered at different adaptation levels. For the size of eye used in these behavioural experiments, the largest receptive field centres found by recording from retina or optic nerve would subtend about 30° of visual angle[16,17], although fields as large or larger that may belong to ganglion cells, are also found in the optic tectum[18,19]. When ganglion cells are recorded in the optic nerve or tectum with the fish's eye under water, the diameters of the smallest field centres are found by different workers to be between 3° and 5° (refs 19–22).

The correspondence between the range of diameters of receptive field centres and critical diameters suggests not only a summing role for ganglion cells, but also that critical diameters in the light and dark are determined respectively by the smallest and largest field centres. To explain this, first assume that the fish responds when one or more ganglion cells signal a significant change in light stimulation. In the fully dark-adapted eye, the largest fields should be the most sensitive for large test stimuli, since they are most likely to catch the minimum number of quanta s^{-1} required for detection. Critical diameter will then be set by the size of these fields. As background luminance is increased, the largest fields will catch more quanta from the background and will, as a result, be more desensitised than smaller fields. This phenomenon has been seen in cat ganglion cells[23]. Critical diameter will then be determined by progressively smaller field centres until, at a background luminance of about 3×10^{-3} mL and above, the smallest fields (3–5° in diameter) will determine the critical diameter. Such a mechanism may be the basis for the recruitment of smaller summing units proposed to account for the improvement of acuity with increasing luminance[1,23].

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Stability of Met-enkephalin content in brain structures of morphine-dependent or foot shock-stressed rats

THE discovery of enkephalin has suggested one more theory to explain the mechanisms of opiate tolerance and addiction. Kosterlitz and Hughes[1] proposed that a reduced enkephalin release might explain tolerance and physical dependence to opiates. Simantov and Snyder[2] supported this view by showing that the content of enkephalin was changed in brain of morphine-dependent rats. Others[3-5] have reported that Met-enkephalin is rapidly metabolised by brain tissue. Because of this *in vivo* lability of Met-enkephalin, particular precaution must be taken in the preparation of brain tissue samples for enkephalin assay. In fact, we have shown that by killing the rats with a microwave beam focused to the head and by taking particular precautions in preparing the brain samples we could measure Met-enkephalin concentrations which were higher (by several orders of magnitude) than the concentrations measured by other authors who failed to take such precautions[6]. Since the changes in the brain enkephalin content reported to occur either after a stress induced by foot shock[7] or during morphine dependence[2], were measured by a binding assay which lacks specificity for enkephalin, we decided to repeat these experiments with a technique that specifically measures Met-enkephalin[6] to decide whether the brain enkephalin content changes during stress[7] or opiate dependence[2].

We duplicated the technique described by Simantov and Snyder[2] to cause morphine dependence and withdrawal, the only deviation from their experimental design being the use of naltrexone 15 μmol kg^{-1} instead of naloxone 1 mg kg^{-1} to elicit morphine withdrawal.

To measure the Met-enkephalin content, male, Sprague–Dawley rats were killed with focused microwave irradiation[8] and the striatum and hypothalamus were dissected as outlined by Glowinski and Iversen[9]. The brain regions were extracted with 0.1 M acetic acid and the extracts neutralised with 1 M NaOH. The assay was carried out in polypropylene tubes. Non-labelled peptide or brain extracts were incubated with antiserum and ^{3}H-enkephalin in 0.5 ml, 0.2 M Tris buffer, *p*H 7.4, containing 0.1% albumin and 0.06% dextran. The ^{3}H-enkephalin bound to antibody was separated from the free ^{3}H-enkephalin by 0.2 ml 1.5% charcoal slurry containing 0.15% dextran and 0.9% NaCl. The supernatant was counted in a Beckman LS 250 liquid scintillation spectrometer. The method of preparation and the specificity of our antibody was as described previously[6].

Table 1 Morphine dependence and withdrawal on the content of Met-enkephalin in various brain structures

Treatment	Met-enkephalin (ng mg^{-1} protein) Hypothalamus	Striatum	Remainder of brain§
Control	2.6±0.9	5.0±1.0	1.0±0.14
Morphine*	3.1±1.5	6.2±2.9	0.91±0.20
Naltrexone†	3.8±0.9	6.3±1.1	0.93±0.15
Morphine‡ and naltrexone	2.8±0.9	7.0±1.5	0.78±0.14

Each value refers to the mean ± s.e.m. of the average of five determinations. The values were not corrected for recovery.

*One morphine pellet prepared according to Gibson and Tingstadt[10] was implanted. After 2 d, two more pellets (75 mg each) were implanted together. Rats were killed with microwave radiation 5 d after implantation of the first pellet.

†Naive rats were injected intraperitoneally with naltrexone 15 μmol kg^{-1} and killed with microwave irradiation 1 h later.

‡Rats were treated as in *, with the exception that naltrexone 15 μmol kg^{-1} was injected 1 h before microwave irradiation and brain extraction.

§The remainder of brain did not contain cerebellum.

As shown in Table 1, naltrexone, implantation of morphine pellets and the combination of the two treatments, failed to change the enkephalin content of various brain regions. The data reported in Table 2 show that after 60 min of intermittent inescapable foot shock administered exactly as described by Akil *et al.*[7] and Madden *et al.*[11], there was no change in the Met-enkephalin content of striatum, hypothalamus or the remainder of brain tissue.

Table 2 Inescapable foot shock on content of Met-enkephalin in various structures

Treatment	Met-enkephalin (ng mg^{-1} protein) Striatum	Hypothalamus	Remainder of brain
Control	8.8±0.6	3.6±0.7	0.43±0.09
Foot shock*	8.1±0.3	2.8±0.3	0.44±0.03

Each value refers to the mean ± s.e.m. of an average of six determinations.

*Foot shock consisted of 60 min intermittent inescapable foot shock (3 mA, 1 s duration, 1 per 5 s). Rats were killed with the microwave radiation immediately after shock.

Our data do not negate the possibility that in conditions of opiate dependence and withdrawal or after unavoidable foot shock, there is change in the concentration of an opiate receptor agonist of the endorphin type. Since our method measures specifically Met-enkephalin, our results merely negate that the brain content of this peptide changes during dependence. It was suggested that this increase in enkephalin was a biochemical reflection of the inactivity of enkephalinergic neurones[2]. It was argued that these neurones had ceased firing when the opiate receptor was occupied by saturating concentrations of morphine. We do not oppose the view that a feedback message links the occupancy of opiate receptors with the rate of firing of enkephalinergic neurones. Our results just indicate that the biochemical signal associated with the cessation of activities in enkephalinergic neurones is not an increase in brain enkephalin content. Perhaps changes in the synthesis rates of the enkephalin have a regulatory role for enkephalin steady state when neuronal activity changes. This possibility is in agreement with the finding that following the injection of naltrexone, the abrupt cessation of opiate receptor occupancy by morphine is not associated with a change in the steady state of Met-enkephalin. In contrast with reports by Akil *et al.*[7] and Madden *et al.*[11], we failed to find an increase in enkephalin content after stress elicited with considerable foot shock. This failure may be due to the more precise methodology that is now possible in comparison with early methods[2,7] or could be due to the fact that the method used by Simantov and Snyder[2] and Akil *et al.*[7] also measured β-endorphin which, during stress, may be mobilised from pituitary and accumulates in brain tissue. Since our method is specific for Met-enkephalin, we failed to detect a possible increase in brain β-endorphin content.

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Comparison of specificity of agonist and antagonist radioligand binding to β adrenergic receptors

DIRECT radioligand binding methods have been used extensively to identify and characterise a wide range of hormone, drug and neurotransmitter receptors. Among the most intensively studied have been the β adrenergic receptors which mediate stimulation of the enzyme adenylate cyclase by catecholamines[1]. Until recently all of the successful binding studies of β adrenergic receptors have been performed with specific high affinity β adrenergic antagonists such as (−) ^{3}H-dihydroalprenolol[2], (±) ^{3}H-propranolol[3] or (±) ^{125}I-hydroxybenzylpindolol[4]. Earlier attempts to use radioligand β adrenergic agonists were generally frustrated by a great deal of nonspecific (that is non-receptor) binding[5]. We have developed a high affinity radiolabelled β adrenergic agonist, ^{3}H-hydroxybenzylisoprenaline, which, when used in conjunction with high concentrations of catechol and ascorbic acid which block non-receptor binding sites, seems to be a useful ligand for studying the β-adrenergic receptors[6]. We present here the results of parallel studies of agonist (^{3}H-hydroxybenzylisoprenaline, HBI) and antagonist (^{3}H-dihydroalprenolol, DHA) binding to β-adrenergic receptors in frog erythrocyte membranes. The ability of sixteen β-adrenergic agents (agonists and antagonists) to competitively inhibit binding of the two ligands in identical assay conditions (including catechol and ascorbate) has been compared. The results suggest an impressive coincidence of the binding data obtained with the two radioligands. These results are in contrast with those recently described for several central nervous system neurotransmitter receptors where it seems to be possible to identify discrete 'agonist' and 'antagonist' conformations of the receptors by performing binding studies with either a radiolabelled agonist or antagonist respectively[7–11].

The sources of all materials used have been reported previously[6,12]. The frog erythrocyte membrane preparation used in these studies was that previously described[13] and was obtained by centrifuging crude frog erythrocyte lysates over a cushion of 50% sucrose at 2,000*g* for 10 min. The membranous material which does not sediment through the sucrose was collected and used in all binding studies. These preparations have a five-to tenfold enhancement of β-receptor binding and adenylate cyclase activity as compared with crude lysates[13]. Binding assays with both radioligands were carried out essentially as before[6,14].

EC$_{50}$ values (concentrations of agents causing 50% displacement of specific binding) for any agent were determined in the same experiment using both radioligands. Five concentrations of each agent were generally tested. We have previously demonstrated that binding of both radioligands reaches steady state within the 10 min incubations at 37 °C (refs 6, 15). Dissociation constants (K_D) of various competing agents were not calculated since such calculations require an estimate of the K_D of the radioligands. We have previously demonstrated that ^{3}H-HBI binding is only slowly reversible and hence Scatchard[16] and similar analyses do not provide meaningful estimates of its affinity for the β receptors.

The ability of the (−) and (+) stereoisomers of propranolol to inhibit the binding of the two radioligands to frog erythrocyte membranes is depicted in Fig. 1*a*. It can be observed that the two sets of curves are virtually superimposable. The result is typical of the displacement curves obtained when β-adrenergic antagonists were used to inhibit radioligand binding

The ability of β-adrenergic agonists to inhibit the binding of the two radioligands is depicted in Fig. 1*b*. These results are typical of the displacement curves obtained with agonists. For potent agonists, such as (±)HBI, the displacement curve for (±)^{3}H-HBI was usually slightly (one-to twofold) to the left of that for (−)^{3}H-DHA. For weak agonists, however, such as (+)adrenaline, the curve for inhibition of (±)^{3}H-HBI was displaced somewhat more to the left of that for (−)^{3}H-DHA (in this case approximately threefold).

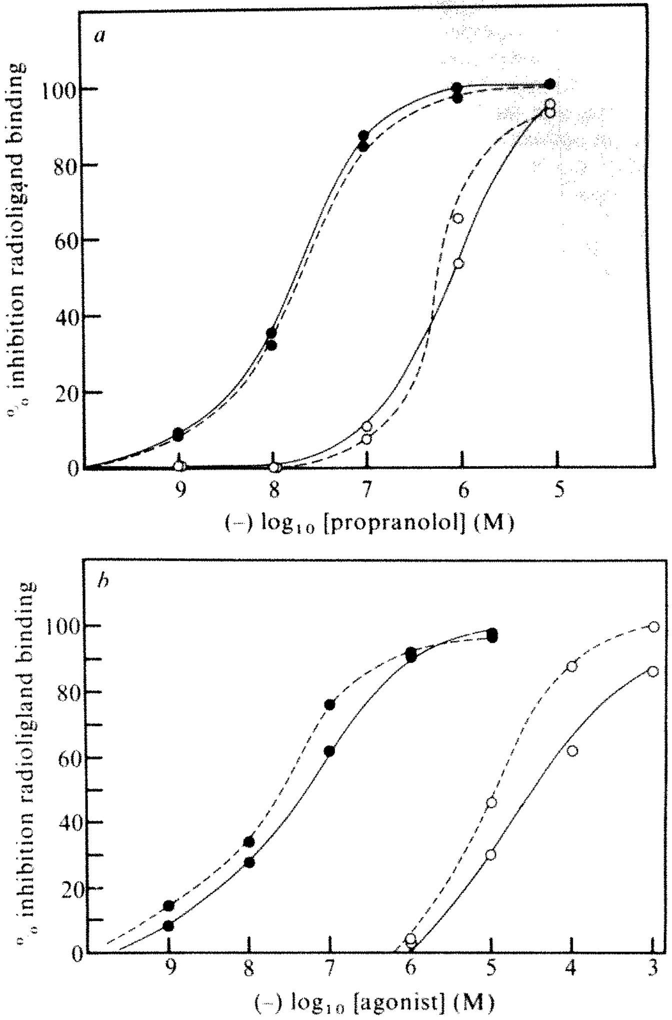

Fig. 1 *a*, Inhibition of radioligand binding to frog erythrocyte membrane β-adrenergic receptors by stereoisomers of propranolol. Membranes were incubated in a final assay volume of 150 μl which contained Tris-HCl, 50 mM (*p*H 7.5), 15 mM MgCl$_2$ and 40,000–50,000 c.p.m. (10 nM) (±)^{3}H-HBI (broken line) or ~ 60,000 c.p.m. (~ 10 nM) (−)^{3}H-DHA (solid line) in the presence or absence of various concentrations of adrenergic agents (—) propranolol (●) and (+) propranolol (○). All assays with either radioligand also contained catechol 3×10^{-4} M and ascorbic acid, 8×10^{-4} M to block non-receptor binding sites which might bind the labelled agonist. All incubations were performed for 10 min at 37 °C and were terminated by vacuum filtration through a Whatman GFC glass fibre filter followed by washing with either 10 ml (^{3}H-DHA) or 20 ml (^{3}H-HBI) of ice cold incubation buffer. Filters were dried overnight then counted in a triton–toluene based fluor in a Packard liquid scintillation spectrometer at an efficiency of 40%. Separate incubations were also carried out in the presence of 10^{-5} M (±)propranolol to determine 'nonspecific' binding. Specific or β-receptor binding in all cases was taken as total minus nonspecific binding. In separate experiments we demonstrated that these washing procedures reduced nonspecific binding while having no effect on specific binding[6,12]. At the radioligand concentrations used in these studies 'specific' binding was routinely ~ 70% of total binding with (±)^{3}H-HBI and ~ 95% of total binding with (−)^{3}H-DHA. Results shown are the means of three experiments. *b*, Inhibition of radioligand binding to frog erythrocyte membrane β-adrenergic receptors by a potent and a weak agonist. Results shown are the means of two or three experiments. Broken line, (±)^{3}H-HBI; solid line, (−)^{3}H-DHA. ●, (±)HBI; ○, (+)adrenaline.

A summary of EC_{50} values for the 16 adrenergic agonists used in this study as inhibitors of ^{3}H-HBI and ^{3}H-DHA binding is presented in Fig. 2 and Table 1. As shown in Fig. 2, the correlation between the two sets of data is excellent. When EC_{50} values for all antagonists for ^{3}H-HBI binding were compared with those for ^{3}H-DHA binding no statistically significant difference was found (paired t test, $P > 0.4$). For agonists, however, there was a significant difference ($P < 0.05$).

It may be noted that some of the EC_{50} values obtained in the present studies, especially those for catecholamine agonists are somewhat lower than values obtained previously[12]. This is apparently due to the protective effect of the catechol and ascorbate in the incubations on these agents and underscores the importance of comparing ^{3}H-HBI and ^{3}H-DHA EC_{50} values in identical experimental conditions.

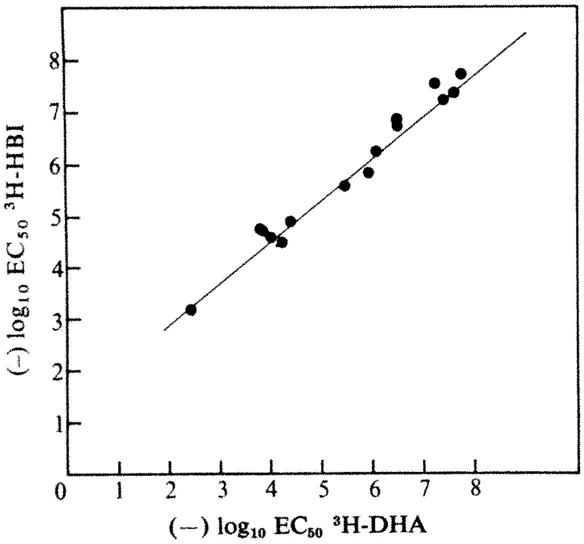

Fig. 2 Correlation of EC_{50} values for β-adrenergic agents determined by inhibition of $(-)^3$H-DHA and $(\pm)^3$H-HBI binding to frog erythrocyte membranes. The line was determined by linear regression analysis. $r = 0.99$.

Table 1 EC_{50} values for β-adrenergic agents determined by inhibition of (−) ^{3}H-dihydroalprenolol and (±) ^{3}H-hydroxybenzylisoprenaline binding to frog erythrocyte membranes

Agent	n	EC_{50} (nM)	
Antagonists		$(-)^3$H-DHA	$(\pm)^3$H-HBI
(−)Propranolol	3	0.018	0.020
(+)Propranolol	3	0.800	0.600
(−)Alprenolol	3	0.025	0.045
(±)Dichlorisoprenaline	3	1.2	1.5
(±)Phenylephrine	2	60	35
Dopamine	2	4,000	700
Agonists and partial agonists			
(±)Hydroxybenzylisoprenaline	3	0.060	0.030
(±)MJ 9184–1	2	0.044	0.060
(−)Isoprenaline	3	0.340	0.180
(−)Soterenol	3	0.320	0.150
(−)Adrenaline	2	3.5	2.5
(−)Noradrenaline	2	100	28
(+)Isoprenaline	2	150	20
(+)Adrenaline	2	42	13
(+)Noradrenaline	2	>1,000	380
(±)Cobefrin	3	150	18

n, Number of experiments determined in duplicate. Inhibition curves were plotted as shown in Fig. 1a and b for all agents for the determination of EC_{50} values.

The widespread distribution of β-adrenergic receptors in mammalian and non-mammalian species, their close relationship with the enzyme adenylate cyclase and the availability of several radiolabelled β-adrenergic antagonist ligands has served to focus a great deal of experimental effort on the β-adrenergic receptors in the past several years[15]. In a number of respects these receptors seem to serve as a prototype of adenylate cyclase-coupled receptors. Unlike the situation with receptors for several polypeptide hormones, there are a wide variety of both agonist and antagonist agents whose interactions with the β receptors can be studied. Until recently, however, only β-adrenergic antagonist radioligands had been successfully used for direct binding studies of these receptors. With the development of $(\pm)^3$H-HBI (ref. 6) it has become possible, for the first time, to perform parallel studies of an adenylate cyclase-coupled receptor with both a radiolabelled agonist and antagonist. As noted above, such studies are of particular interest in view of recent reports that for several central nervous system neurotransmitter receptors, discrete 'agonist' and 'antagonist' states of the receptors can be identified with such radioligands[7–11]. In such studies it has generally been found that agonists have considerably higher affinity (one to three orders of magnitude) for the sites labelled with the radiolabelled agonist, whereas antagonists have much higher affinity for sites labelled with radiolabelled antagonists.

The data presented here apparently indicate a somewhat different situation for the adenylate cyclase-coupled β-adrenergic receptors of frog erythrocytes. Binding displacement curves using the radioligand agonist and antagonist seem to be in close agreement. A slight but systematic and statistically significant difference does exist for weak agonists which are somewhat (two-to eightfold) more potent in displacing the labelled agonist than the antagonist. The significance of this finding is not clear at present.

These findings do not exclude the possibility that there may be separate 'agonist' and 'antagonist' conformations of the β-adrenergic receptors in frog erythrocyte membranes. If such do exist, however, our data indicate that they are freely interconvertible in the membranes and in the assay conditions used in these experiments. These observations are in marked contrast to several other receptor systems noted above, wherein agonist and antagonist binding give strikingly different results[7–11].

The ability to study the adenylate cyclase-coupled β-adrenergic receptors with both agonist and antagonist radioligands should provide new insights into the nature and regulation of these receptors.

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Development of β-adrenergic receptor subsensitivity by antidepressants

CONSIDERABLE evidence exists that the tricyclic antidepressant drugs are selective inhibitors of monoamine uptake into monoaminergic nerve terminals[1,2]. These drugs also potentiate pharmacological responses to the monoamines in both the peripheral and central nervous systems[3–6]. These effects do not explain the discrepancy between the time-course of the biochemical and pharmacological responses which are elicited by these antidepressant drugs within minutes or hours and their clinical antidepressant action which requires treatment for weeks for efficacy to be reached. Furthermore, desipramine, an antidepressant drug, can potentiate neuronal responses to noradrenaline and dopamine in the caudate nucleus[7], although it does not block the catecholamine uptake in this area of the brain[1,2]. Finally the antidepressant iprindole does not influence noradrenaline turnover[8] or metabolism[9,10] or its uptake into catecholaminergic neurones[11], but is effective in potentiating the responses of single cortical and caudate neurones to monoamines[12]. Vetulani and his associates[13] suggested that the therapeutic action of tricyclic antidepressants may be related to postsynaptic adaptive changes in the sensitivity of the noradrenergic adenylate cyclase receptor system rather than to acute presynaptic events. The purpose of the experiments described here was to explore the molecular basis for antidepressant-induced noradrenergic subsensitivity by examining the kinetic properties of the β-adrenergic receptors in the microsomal suspension of rat brain by using a potent β-adrenergic receptor antagonist ^{3}H-dihydroalprenolol as a radiolabelled ligand[14–19]. Our results indicate that the main mechanism is a reduction in the number of receptors.

Specific ^{3}H-dihydroalprenolol binding to the microsomal fraction obtained from brain in control and in desipramine, iprindole- and doxepin-treated rats is shown in Table 1. When the concentration of ^{3}H-dihydroalprenolol was 4 nM, the specific binding to the control preparation was found to be 70 pmol per g protein. Chronic treatment of rats with desipramine, iprindole and doxepin decreased the specific binding by 65.5%, 42.3% and 30.6% as compared to control, respectively. This observation suggests that the resistance of adenylate cyclase to noradrenaline following chronic administration of antidepressants is due to subsensitivity of noradrenergic β-receptors. This decreased sensitivity of β-adrenergic receptors towards actecholamines following chronic administration of antidepressants may be due to alterations of the apparent affinity of these receptors to dihydroalprenolol or to a decrease in the number of β-adrenergic receptor sites. The dissociation constants of ^{3}H-dihydroalprenolol and the densities of the β-adrenergic receptors in control and desipramine-treated rat brain membrane fractions were determined by measuring the specific binding of various concentrations of ^{3}H-dihydroalprenolol and analysing the data by the method of Scatchard[20]. Only a single class of high affinity binding sites (Fig. 1) for ^{3}H-dihydroalprenolol could be detected in rat brain microsomes.

Table 1 Effect of chronic administration of antidepressants on specific dihydroalprenolol binding

Drug treatment	Specific ^{3}H-dihydroalprenolol binding pmol per g protein
None	70.0±3.5
Desipramine	24.2±1.5 ($P < 0.001$)
Iprindole	40.4±2.8 ($P < 0.001$)
Doxepin	48.6±2.4 ($P < 0.001$)

Desipramine, iprindole and doxepin were injected intraperitoneally in male, Sprague–Dawley rats, weighing 180–220 g, at a dose of 10 mg per kg body weight daily for 6 weeks. The rats were killed 1 h after the last injection by decapitation and the brains were rapidly removed and chilled in ice-cold 0.9% NaCl. The whole brain minus cerebellum was homogenised using a Brinkman Polytron, in 20 volumes of ice-cold 0.05 M Tris buffer (pH 8.0 at 25°), and centrifuged at 49,000*g* for 15 min. The pellet was rehomogenised in the same buffer and centrifuged as before. The pellet was finally resuspended in 50 volumes of Tris buffer (pH 8.0 at 25°) and used fresh for the binding assay. ^{3}H-dihydroalprenolol (32 Ci mmol^{-1}) binding was determined at 23 °C by combining 0.97 ml of the above particulate suspension, 4.0 nM ^{3}H-dihydroalprenolol and sufficient distilled water to make 1 ml as the final volume of the reaction mixture. After 20 m in incubation, the reaction mixtures were filtered under reduced pressure through Whatman glass fibres (GF/B). The filters were rinsed four times with 4 ml ice-cold Tris buffer to remove most of the unbound radioactive ligand. The filter papers were dried and placed in a counting vial containing 10 ml of Scintiverse (Fisher) and counted in a Packard Tri-carb liquid scintillation spectrometer (Model 3380) at 30% efficiency. Corrections were made for nonspecific accumulation of radioactivity by assaying parallel incubations which contained a large excess (100 μM) of noradrenaline. Specific binding, defined as the difference between total and nonspecific radioactivity was 60–70% of the total binding. Values are means ± s.e.m.

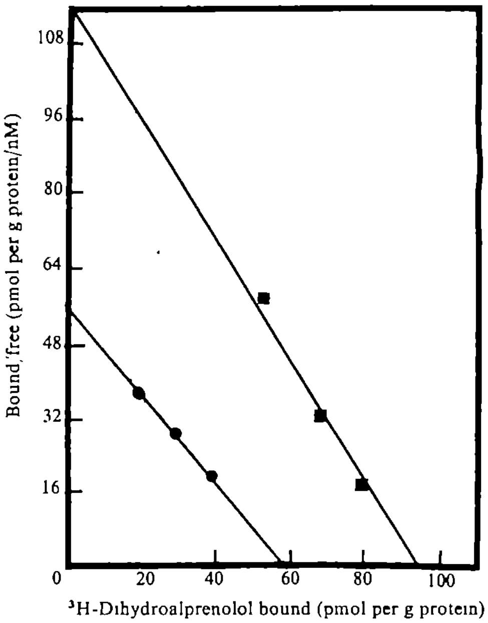

Fig. 1 Scatchard analysis of specific ^{3}H-dihydroalprenolol binding in brain microsomal fractions of control (■) and desipramine (●) -treated rats. The concentrations of ^{3}H-dihydroalprenolol used varied from 0.5 to 4.0 nM. (Procedure as for Table 1.) Dissociation constant and maximal number of binding sites were calculated from this figure and given in Table 2.

This observation is in agreement with the previously reported results of Bylund and Snyder[19]. The dissociation of ^{3}H-dihydroalprenolol binding to control rat brain particulate fraction was found to be 0.82 nM and this value is similar to the affinity constant (1.3 nM) of β-adrenergic receptors to ^{3}H-dihydroalprenolol reported by Bylund and Snyder[19]. Although the dissociation constant of ^{3}H-dihydroalprenolol to β-adrenergic receptors did not increase significantly, the density of receptors markedly decreased following chronic administration of desipramine (Table 2). This would suggest that chronic administration of desipramine leads to a decrease in the density of β-adrenergic receptors.

There are several lines of evidence indicating that modification of the presynaptic activity of noradrenaline, by altering, its rate of synthesis, release, uptake or storage, causes changes at the postsynaptic sites which are manifested as supersensitivity or subsensitivity. Such alterations in the sensitivity of the postsynaptic receptors for noradrenaline have been demonstrated by measuring noradrenaline-stimulated accumu-

Table 2 Scatchard analysis of specific ^{3}H-dihydroalprenolol binding to brains of control and chronically desipramine-treated rats

Brain preparation	Dissociation constant (nM)	Density of receptors (pmol per g protein)
Control	0.82 ± 0.08	95 ± 5
Desipramine treated	1.09 ± 0.14	59 ± 3
	$(P > 0.05)$	$(P < 0.001)$

The dissociation constants of ^{3}H-dihydroalprenolol and the density of binding sites were determined by Scatchard analysis[20]. The procedures for the preparation of the particulate fraction and measurement of specific binding of ^{3}H-dihydroalprenolol to membrane fractions are described in Table 1. Each experimental point is the average of nine determinations obtained from three animals in each group.

lation of cyclic AMP after administration of drugs such as 6-hydroxydopamine, cocaine and reserpine[21-23]. In general, drugs that decrease the activity of noradrenaline at the presynaptic sites cause supersensitivity as opposed to drugs that produce subsensitivity by increasing noradrenaline levels at the synaptic cleft. Vetulani and his associates[13] have provided evidence that chronic administration of antidepressants leads to subsensitivity of noradrenaline stimulated adenylate cyclase. Our results suggest that this resistant state of adenylate cyclase towards noradrenaline after chronic administration of antidepressants is due to a decrease in the number of β-adrenergic receptors. A hypothesis that β-adrenergic catecholamines are able to regulate catecholamine sensitivity of tissues *in vivo* by regulating the number of β-adrenergic receptors has been advanced[24]. The effect of desipramine provides some support to the above hypothesis. On the other hand, iprindole, with a minimal effect at presynaptic neurones, caused β-adrenergic receptor subsensitivity (Table 1).

The results suggests that although the level of noradrenaline may have an important role in controlling β-adrenergic receptor density, the concentration of this neurotransmitter at the synaptic cleft is not the only mechanism for the regulation of β-adrenergic receptor concentration.

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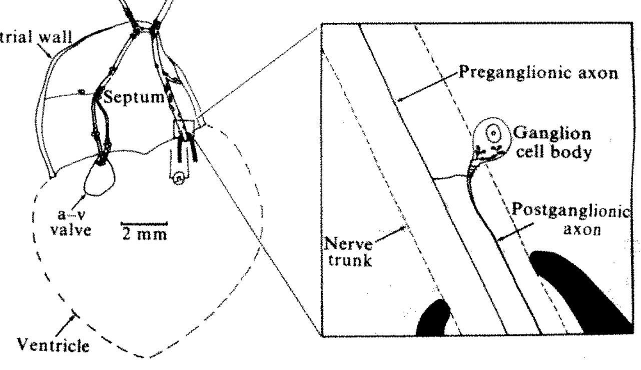
Formation of synapses between parasympathetic neurones deprived of preganglionic innervation

MUSCLE fibres will accept innervation from a foreign nerve only if synaptic transmission from the appropriate nerve has been interrupted[1]. The native nerve supply thus exerts an influence which renders muscle resistant to abnormal innervation. Do such influences act to prevent neurones from receiving inappropriate synapses? In the central nervous system (CNS) surviving inputs to partially denervated neurones will sprout to form additional synapses on those same neurones[2-4]. Furthermore, in partially denervated autonomic ganglia, surviving inputs will sprout to form synapses on postganglionic neurones they did not innervate originally[5-7]. But there has been no electrophysiological demonstration that denervated neurones will spontaneously receive synapses from a class of nerve cells with which there is normally no contact. We report here that the parasympathetic ganglion cells of the frog heart form functional synaptic connections with each other when their preganglionic synaptic input is removed; these intraganglionic connections do not occur normally.

The parasympathetic ganglion cells of the frog heart lie along two major nerve trunks in the interatrial septum (Fig. 1*a*)[8]. These trunks, which are extensions of vagal nerve branches, enter the heart at the sinus venosus, traverse the septum and pass into the ventricle. Stimulation of the nerve trunks at the point where they pass into the ventricle (Fig. 1*b*) results in antidromic activation of most ganglion cells in the septum (see Fig. 2*a*).

We searched for synaptic connections between ganglion cells by stimulating the nerve trunks antidromically and recording intracellularly from individual cells. In normal ganglia this procedure is complicated by the fact that synaptic potentials may result also from antidromic stimulation of preganglionic axons, branches of which sometimes extend as far as the ventricular edge of the septum (Fig. 1*b*)[9]. This complication was avoided by carrying out all experiments 2 d or more after vagotomy, by which time preganglionic transmission has ceased. The hearts of *Rana pipiens* were denervated by bilateral resection of the vagus nerve[10], and reinnervation was delayed

Fig. 1 Schematic view of the frog interatrial septum. *a*, Ganglion cell bodies (not drawn to scale) are seen along the two nerve trunks that pass through the septum. One of the a–v valves has been removed, and the nerve trunk has been drawn into a suction electrode (stippled) for antidromic stimulation of ganglion cells (see expanded view in *b*).



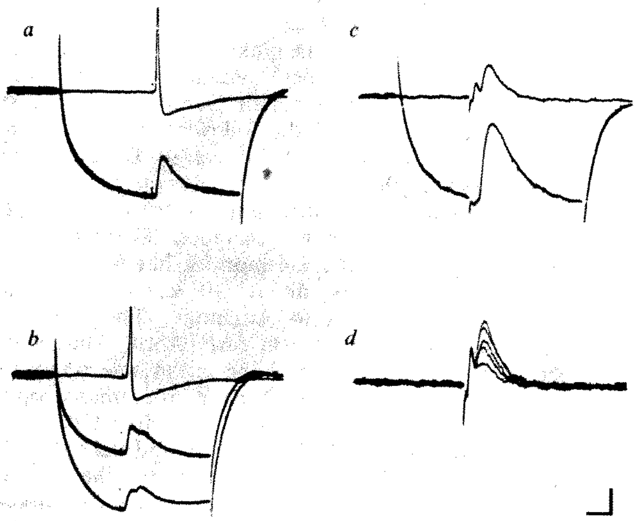


Fig. 2 Action potentials and synaptic potentials recorded intracellularly from frog cardiac cells. *a*, An antidromic action potential was elicited by nerve trunk stimulation (upper trace). When the cell was hyperpolarised by current injected through the recording electrode, the action potential failed to invade the cell body and appeared instead as a monotonic depolarisation (lower trace). This neurone was denervated 4 d previously to allow degeneration of the preganglionic fibres. *b* And *c*, both action potentials and synaptic potentials were elicited from ganglion cells deprived of preganglionic input for 2–3 months. *b*, Neurone responding to antidromic stimulation with an action potential, apparently without a synaptic potential (upper trace). When the cell was hyperpolarised by current passed through the recording electrode, however, a small synaptic potential was seen on the falling phase of the blocked action potential (middle trace). Further hyperpolarisation reduced the amplitude of the blocked action potential and enhanced the synaptic potential (bottom trace). *c*, Antidromic stimulation elicited an action potential that did not invade the cell body and a synaptic potential. The action potential was not observed on hyperpolarisation and the synaptic potential was enhanced. *d*, The amplitude of this same synaptic potential fluctuated in response to repeated stimuli of constant intensity in a manner characteristic of low quantal content synaptic transmission (five stimuli, delivered at 0.3 Hz). Horizontal calibration 20 ms; vertical calibration, 20 mV in *a* and *b*, 10 mV in *c*, 5 mV in *d*. Experiments were carried out in Leibovitz-15 medium diluted to 40% and supplemented with inorganic salts, glucose and buffer to give the following concentrations (in mM): NaCl, 114; KCl, 2.1; $CaCl_2$, 3.6; $MgCl_2$, 0.8; glucose, 5; HEPES, 5; *p*H, 7.2. Electrophysiological recordings were made with micropipettes filled with 4 M potassium acetate and bevelled to resistances of 60–120 MΩ. Only cells which had resting potentials more negative than −40 mV were studied.

by a second resection 50–60 d after the first. In experiments carried out long after denervation, the possibility of vagal reinnervation was checked by orthodromic stimulation of the vagus nerves; septa which had been reinnervated were not used.

Synaptic potentials elicited by antidromic nerve stimulation were not detected in septa denervated for short times. In nine animals examined 2–5 d after denervation, no synaptic potentials were encountered in 200 neurones examined (175 responded with antidromic action potentials). We conclude that intrinsic synaptic connections are absent in normally innervated septa. In contrast, however, in seven septa denervated for 60–100 d, antidromic stimulation elicited synaptic potentials from 52 of 107 neurones examined (93 responded with antidromic action potentials). No significant difference in resting potentials or input resistance was noted between these cells and those denervated for short times. Sample recordings from two neurones that show both antidromic action potentials and synaptic potentials are reproduced in Fig. 2*b* and *c*. The synaptic origin of these potentials is indicated by the fact that they were enhanced by hyperpolarisation, that they were abolished by 10^{-4} M *d*-tubocurarine, and that their amplitudes often showed fluctuations characteristic of low quantal content transmission (Fig. 2*d*).

Two kinds of experiments were carried out to show that the synaptic potentials recorded in denervated ganglia arise from connections between the ganglion cells. In the first, to demonstrate synaptic coupling between pairs of ganglion cells, an intracellular recording was made from one neurone while the cell bodies of neighbouring neurones were stimulated extracellularly (see Fig. 3*a*). The probability of success of this experiment was low since usually less than 2% of the cells in the septum were accessible for stimulation within a single field of view (diameter 400 μm). In 2 of 388 pairs tested, extracellular stimulation of one ganglion cell produced a synaptic potential in another (Fig. 3*b*). In the second group of experiments, we searched for synaptic potentials in septa which had been denervated chronically and then isolated and maintained *in vitro*. Since parasympathetic ganglion cells are the only neurones whose cell bodies lie within the septum, only these cells should remain viable in isolated septa. Indeed, when normal heart septa were cultured at 22 °C, preganglionic synaptic transmission failed in 24–36 h. In contrast, synaptic potentials could be elicited by antidromic nerve trunk stimulation from many neurones in septa denervated for 3 months and then maintained *in vitro* for 3–4 d (14 examples in 46 cells examined; culture medium as described in Fig. 2 legend, supplemented with foetal calf serum and antibiotics). We conclude that the synaptic potentials elicited in isolated septa result from synaptic interaction between ganglion cells.

Our results indicate that prolonged loss of normal preganglionic input leads to the formation of excitatory synapses

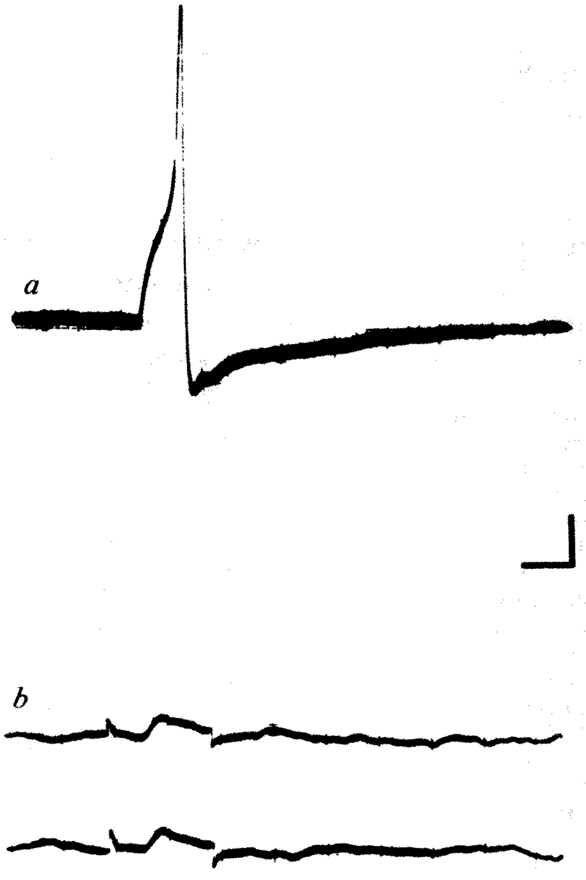


Fig. 3 Direct demonstration of synaptic interaction between ganglion cells. *a*, Stimulation by a blunt extracellular micropipette elicited an action potential from a ganglion cell. *b* (a different experiment), three successive e.p.s.ps recorded from a neurone in response to extracellular stimulation of another nerve cell 300 μm distant (40-ms stimulation pulses). Calibration: 10 mV, 20 ms.

between frog parasympathetic neurones. These postganglionic collateral synapses do not exist normally in frog, whereas they do in the mudpuppy cardiac ganglion[11,12] and in the superior cervical ganglion of guinea pig[7]. These connections also develop between dissociated rat sympathetic ganglion cells in culture[13,14]. The capacity to form collateral synapses in frog presumably arises in part because acetylcholine is both the pre- and the postganglionic transmitter in the cardiac ganglion. Experiments are now in progress to determine the fate of these intraganglionic synapses following reinnervation of ganglion cells by their normal vagal inputs.

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Synaptic and septate neuromuscular junctions in embryonic lobster muscle

IT is generally accepted that there is not enough genetic material to programme uniquely the structure of each neurone in multicellular animals. This then presents the problem of determining how a growing neurone finds and identifies the correct postsynaptic structures. Work on the visual system of *Daphnia* has demonstrated that the primary sensory neurones form transient gap junctions with undifferentiated neuroblasts in the optic lamina[1]. No similar work has been reported for interneurones or motor neurones, however. For instance, we do not know how developing motor neurones find the proper muscle in the periphery[2]. I studied neuromuscular development in lobster embryos and report here that motor neurones form septate junctions with developing muscle cells in the abdomen. In addition, mature neuromuscular synapses are present in the embryo as early as the third to fourth month of the total 9 month development.

Crustacean neuromuscular systems offer several advantages for studying developmental processes. Each muscle is innervated by a few identifiable neurones, at least one of which is inhibitory. Muscle fibres each receive multiple inputs, but not all fibres within a muscle are identically innervated[3,4]. For these reasons, it has been suggested that these systems might serve as useful models to study processes which occur in central nervous systems[5]. Previous work on developing arthropod neuromuscular systems has been limited primarily to studies on insect larvae[6,7]. One ultrastructural study on developing crayfish opener muscle suggested that active synapses were not present just before hatching[8].

Egg-bearing lobsters (*Homarus americanus* Milne-Edwards) were trapped in local waters around Woods Hole, Massachusetts, and kept in tanks of running seawater at ambient temperatures. These animals extrude and fertilise the eggs during September or October, then brood them under the abdomen until the following May or June, when the larvae all hatch within 1–2 d (ref. 9). Development is not constant during this period; after extrusion, development proceeds for several months until water temperature falls below about 10 °C during December. There is little development for the following 5–6 months, but when water temperature again reaches about 10 °C, during late April, development proceeds to hatching[10]. The embryos used in this study were removed in early April. Abdomens were isolated from the thorax and the telson was removed to permit penetration of fixative for electron microscopy. Fixation was in 2% glutaraldehyde, 1% formaldehyde, 100 mM cacodylate at *p*H 7.4, 4% sucrose and 0.5% NaCl. Manganese chloride (0.1%) was added to the initial fixative to prevent release of synaptic vesicles during fixation (unpublished). Sections were cut on a diamond knife and post-stained with uranyl acetate and lead citrate.

The abdominal muscles do not differentiate synchronously during development. In all embryos fixed between December and April it was possible to observe fully differentiated short (1.5–2.5 μm) and long (2.5–4.0 μm) sarcomere muscle fibres as well as undifferentiated muscle cells. Synaptic profiles, which were generally similar to those seen in adult muscle, were observed only on mature muscle fibres (Fig. 1). Growing axons were characterised by the presence of two populations of vesicles, one with a diameter of about 40–50 nm and the other a diameter of about 60–90 nm (Figs 2 and 3). The former are the same size as the vesicles normally observed in lobster excitatory synapses. But, in the growing axons many of the vesicles were electron opaque while those in neuromuscular synapses are agranular. The second, larger type of vesicle is similar to the dense-cored vesicles of unknown function which have been described in a wide variety of neuromuscular synapses[11].

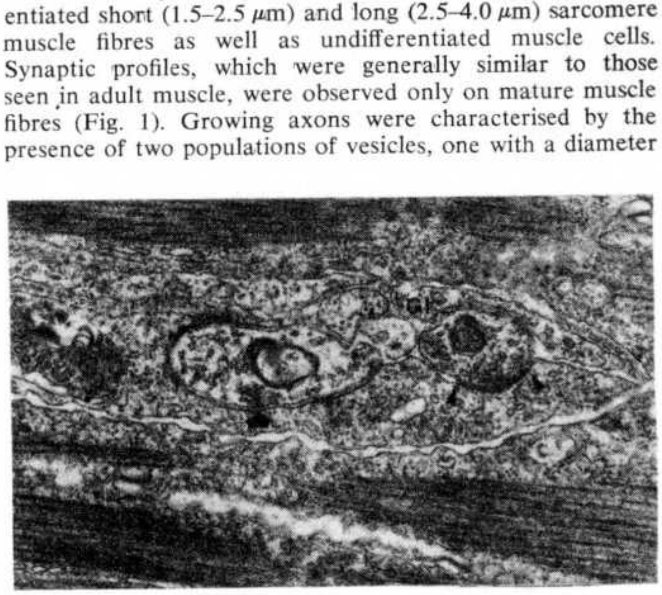

Fig. 1 Excitatory and inhibitory neuromuscular junctions on embryonic lobster muscle. The excitatory terminal (right) has the characteristic round synaptic vesicles. The synaptic region has a heavily stained pre- and postsynaptic membrane (between arrowheads). Densely staining material is present in the synaptic cleft. The inhibitory terminal (left) contains smaller, irregular vesicles and the pre- and postsynaptic membranes are characteristically not heavily stained (see ref. 8). A presynaptic dense body, with vesicles clustered around it, is present (arrow). These nerve terminals are on a long sarcomere fibre; similar terminals are observed on short sarcomere fibres. (×28,200.)

The growing ends of the axons are always observed in proximity to embryonic muscle cells and are characterised by several features. First, vesicles are invariably present in rather high densities. Second, growth cones or 'microspikes'[14] are present and are packed with vesicles (Figs 2, 3). Third, a number of tubular structures are

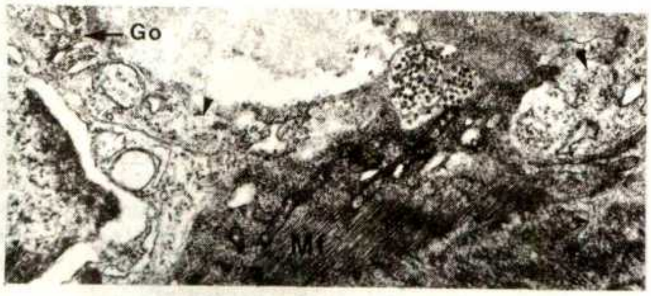

Fig. 2 Growth cone of a motoneurone in abdominal muscle of embryonic lobster. The growth cone is characterised by the presence of numerous vesicles ranging from about 50–90 nm in diameter (see text). The cone, which is adjacent to an embryonic myotubule, appears to be in the process of dividing. Note microtubules (arrowheads), Golgi apparatus (Go) and myofilaments (Mf) in the myotube. (×9,000.)

present. Some of these seem to be neurotubules but others enlarge into vesicular structures and may represent some type of endoplasmic reticulum. These axons, unlike mature motor axons, are never covered with glial cells.

The muscle cells that are associated with the growing axons have a distinctive appearance different from mature muscle fibres. They resemble the myotubes described for developing vertebrate muscle[12]. There are never any sarcomeres present but near the border there is always a band of myosin with interdigitating actin filaments (Figs 2, 3). This band is often adjacent to an area of very dense sarcoplasm. The borders of the muscle cell always contain microtubules and a granular substance, probably glycogen. Microtubules are present in mature muscle fibres, but never in the density observed in these embryonic muscle cells, except at the tendonal ends (unpublished, and see ref. 13).

A small process of a growing neurone has been observed to form a septate junction[15] with a muscle cell, possibly a myoblast (Fig. 4). The neurone is characterised by the presence of numerous vesicles. The other structure has features characteristic of embryonic muscle cells; the granular sarcoplasm contains mitochondria and microtubules. It is unlikely to be a glial cell as these have not been observed around the growing ends of the motor axons. In any case, septate junctions are not common features in the embryonic neuromuscular system. In the thirty preparations examined, extensive portions of growing axons were observed in five specimens, lesser portions in two other specimens. Only two septate junctions have been observed, however; thus these connections may be rare, transient[1] or both.

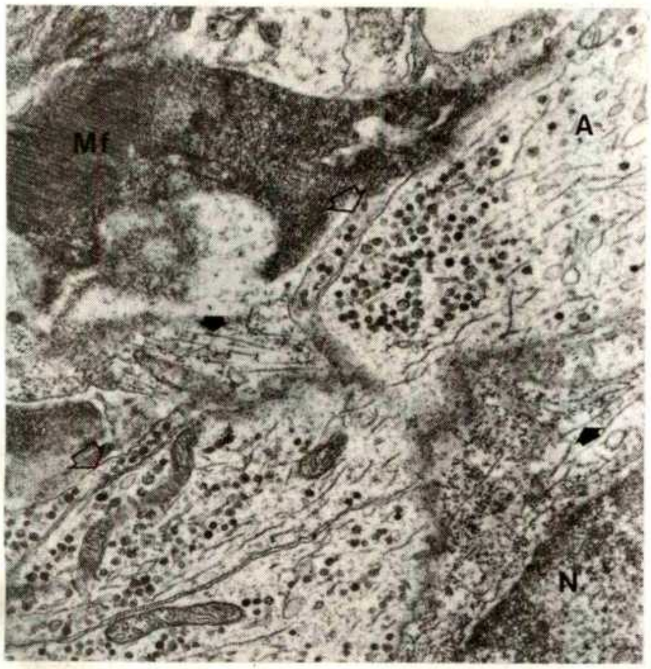

Fig. 3 Growing region of axons (A) which is overlying an embryonic muscle cell. Two 'microspikes', filled with vesicles, are present (open arrows). The muscle cell nucleus (N) is at the bottom right. The sarcoplasm contains microtubules (filled arrows) and glycogen. A band of myofibrils (Mf) is present in the upper left. (×16,625.)

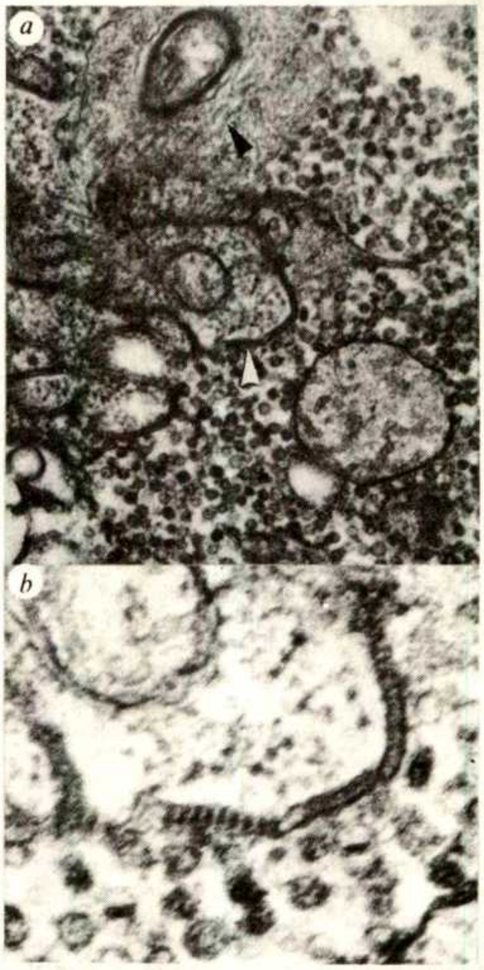

Fig. 4 Septate junction between a growing motoneurone and an embryonic muscle cell, probably a myoblast. The muscle cell has the characteristic granular sarcoplasm and microtubules (dark arrowhead). The neurone is filled with vesicles and has several small projections. *b*, An enlargement of the septate junction (white arrowhead) shown in the middle of *a* figure. (*a*, ×31,540; *b*, ×92,960.)

These results demonstrate that lobster motor axons can have two types of connections with muscle during embryogenesis. The growth cones of growing axons form septate junctions with immature muscle cells. This might well be a period when the two different cell types can exchange chemical information. Later, when the muscle fibres have differentiated to a stage where sarcomere formation is well advanced, mature synaptic endings can be observed. Intracellular recording from these muscles has revealed that the synapses are indeed active. As early as the third month of development miniature excitatory postsynaptic potentials (e.p.s.ps) can be observed. Occasional spontaneous e.p.s.ps and inhibitory postsynaptic potentials have also been observed (in preparation). Thus it seems that both growth cones and active synapses may be present on these motor axons at the same time.

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Is recombination confined to structural genes on the eukaryotic genome?

THE size of the eukaryotic genome increases with the evolutionary complexity of the organism, although this relation is somewhat obscured by large differences in the haploid DNA content of closely related species. Yet the total number of genes is believed to be fairly constant, suggesting that genes of higher eukaryotes correspond to much larger DNA segments than in microorganisms[1]. In that case, only a small fraction of each gene would code for the ultimate gene product, the remainder being 'spacer' DNA with still ill-defined functions. The sequence coding for the gene product will be hereafter designated as the structural gene. The complex organisation of chromatine in higher organisms raises the problem of its possible influence on the distribution of recombination events. I shall present here evidence suggesting that they may well be restricted to small regions of the genome, possibly corresponding to the structural genes.

The basic argument is that the length of total chromosome maps is fairly constant among eucaryotes (Table 1, Fig. 1) and that the same applies to intragenic fine-structure maps among the few organisms where they have been extensively investigated (Fig. 1*b*) such as several fungi, *D. melanogaster*, rice and maize. The conservation of the total recombinational length of eucaryotic genomes means that the average frequency of crossover per unit length of DNA decreases by several orders of magnitude from lower to higher eukaryotes (Table 1, column 4). As opposed to intergenic crossover, intragenic recombination is mainly a non-reciprocal exchange. Yet, both seem to be related processes resulting from the formation of hybrid DNA molecules[2,3]. There are theoretical grounds to believe that they are either in the same, or in a 1/2 ratio with respect to actual distances[4]. If one further assumes that exchanges are randomly distributed on the genome and that intragenic maps correspond to the structural gene even in higher eukaryotes, the length of intragenic maps should considerably decrease from lower to higher eucaryotes.

The comparison of intragenic maps is complicated by two factors. First, recombination within a gene is not always proportional to actual distances, because of marker effects which are more or less prevalent depending on the organism investigated. Second, different regions of the genome have a different density of exchange per unit length of DNA, resulting in marked heterogeneities in the length of intragenic maps within one organism (Fig. 1*b*). On the whole, however, the length of intragenic maps are roughly comparable in all eukaryotes investigated (Table 1, column 2 and Fig. 1*b*).

A key issue here is whether intragenic maps correspond to DNA stretches of comparable length in all eukaryotes. If intragenic maps were to include not only the structural genes but also the surrounding spacers, they would correspond to comparatively long DNA fragments in higher eukaryotes. This would compensate for the general decrease in the average frequency of recombination per unit length of DNA, and may result in the recombinational length of genes being fairly constant for all organisms. However, evidence suggests that point mutations in the spacers do not, as a rule, prevent gene expression (in other words, that intragenic maps are limited to the structural genes).

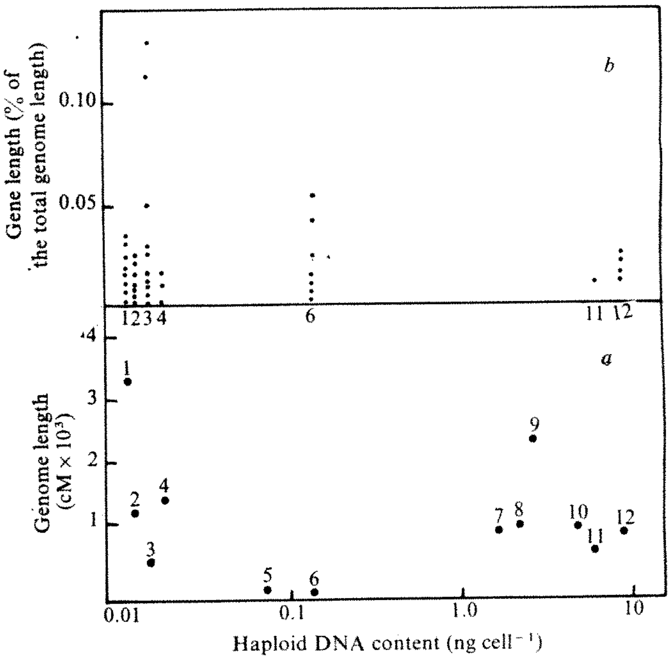

Fig. 1 Recombination frequencies as a function of haploid DNA content. (*a*) Total genome length in centimorgans (cM). (*b*) Length of individual intragenic maps as a fraction (per cent) of the total genome length. Each point represents an individual gene map as given in Table 1, column 2. Haploid DNA content from refs 9, 10 and 11, except for *N. crassa*[39], *A. nidulans*[30], *C. radiatus*[31], *C. elegans*[23], *O. sativa*[32] and *P. sativum*[40]. The organisms represented are: *S. cerevisiae*[1], *S. pombe*[2], *N. crassa*[3], *A. nidulans*[4], *C. elegans*[5], *D. melanogaster*[6], *L. esculentum*[7], *M. musculus*[8], *H. sapiens*[9], *P. sativum*[10], *O. sativa*[11], and *Z. mays*[12].

First, it is established that spacers of rRNA evolve much more rapidly than the structural loci themselves[5] indicating, in that case at least, that there is no stringent requirement imposed on the spacer sequence. Second, work on the *rosy* locus of *D. melanogaster*[8] has shown full coincidence between an intragenic map constructed on 'null' mutants and another based on mutants with altered electrophoretic properties in the gene product, that is, mutants defining the structural locus. Similar evidence is available at two other loci of *D. melanogaster*[7,8]. Finally, the frequency of spontaneous forward mutation per locus per gamete is similar for yeasts, *D. melanogaster*, and mouse (Table 2) which suggests again that genes (defined as loci mutable to non-complementing alleles with a null phenotype) correspond to the same length of DNA in all eukaryotes.

On the other hand, the frequency of forward mutation per locus increases with the size of the genome when mutation is induced by X rays[9] or by ethyl methane sulphonate[10] (see ref. 11 for a critical evaluation of these data). X rays and perhaps EMS-induced intralocus mutants, however, are often deletions or equivalent multisite events in higher eukaryotes, whereas they are exclusively point mutations in fungi (see ref. 12). X rays do induce deletions in fungi, but these are multilocus events covering

Table 1 Total linkage maps and intragenic maps among eukaryotes*

Organism	Length of intragenic maps (cM)		Total length	Average frequency of intergenic recombination (cM × kb⁻¹)
Saccharomyces cerevisiae (budding yeast)	*ura*2-A	0.12[21]	3,600	0.26
	*ura*2-C	0.25[21]		
	*ade*3	0.80		
	*arg*4	1.16		
	*ade*8	1.25		
	*his*1	0.36		
	*arg*B	0.37[22]		
	*arg*C	0.60[22]		
Schizosaccharomyces pombe (fission yeast)	*ade*1	0.05	1,300	0.08
	*ade*2	0.04		
	*ade*4	0.13		
	*ade*6	0.05		
	*ade*7	0.10		
	*ade*8	0.01		
	*ade*9	0.35		
	*his*2	0.01		
	*aro*3	0.01		
	*try*1	0.28		
	*try*2	0.18		
	*try*3	0.05		
	*lys*1	0.04		
	*cdc*2	0.04		
Aspergillus nidulans	*ade*8	0.16	1,600	0.03
	*paba*1	0.05		
	cho	0.30[37]		
Neurospora crassa (bread mould)	*trp*1	0.82	600	0.05
	*trp*3	0.08		
	cys	0.30		
	*pan*2	0.70		
	*met*2	0.10		
	am	0.02		
	*ade*8	0.17		
	*pyr*3	0.03		
	arom	0.18		
	*his*3	0.06		
Ascobolus immersus	75	3.0		
	84*w*	1.2		
	46	0.6		
	*w*17	0.4		
Sordaria fimicola	*G*	0.04		
Coprinus radiatus and *lagopus*	*ura*1	0.03		
	ftr	0.15		
Caenorhabditis elegans			360	0.004
Tetranychus sp. *Acarina* (spider mite)	*albino*	0.20[24]		
Drosophila melanogaster (fruit fly)	*Notch*	0.14[25]	275	0.0017
	dp	0.14[25]		
	r	0.01[26]		
	bx	0.03[25]		
	w	0.026[25]		
	garnet	0.01[36]		
	rosy	0.005[16]		
	ma-1	0.001[7]		
Lycopersicon esculentum (tomato)			1,100	0.0006
Mus musculus (mouse)			1,200	0.0006
Homo sapiens (man)			2,600	0.0009
Pisum sativum (pea)			1,200	0.0002
Oryza sativa (rice)	*glt*	0.10[27]	750	0.0001
Zea mays (maize)	*adh*1	0.11[28]	1,100	0.0001
	wx	0.25[14]		
	*gl*1	0.12[15]		
	*su*1	0.20[29]		

*In most cases, roughly linear maps can be constructed on the basis of two-factors crosses, thus supporting the assumption that intragenic frequencies of recombination are proportional to the actual distances on the gene and providing a direct estimate of the recombinational length of the gene. When necessary, recombination frequencies have been corrected for map expansion[4]. In some cases, the intragenic order of the alleles is quite ambiguous, and the recombinational length of the gene was assumed to be given by the heteroallelic combination giving the highest frequency of recombinant, though this need not define the largest distance in terms of base pair number. Total genome maps from ref. 20 except for *S. pombe*[34], *N. crassa*[18], *M. musculus*[33] and *H. sapiens*[35]. Except when not explicitly given, references for intragenic fungal maps are to be found in refs 2, 18 and 19, whereas data for *S. pombe* are either quoted in ref. 20 or come from unpublished work of Leupold and co-workers.

at least two genes and recoverable as recessive lethals in *N. crassa* dicaryons[13]. Thus, the differences in mutation rate could be due to deletions being retained as intralocus events as long as they remain within the boundaries of a single band in higher eukaryotes, whereas they are selected against in microorganisms where intercistronic regions are quite short.

Hence, it can be assumed that even in higher organisms the actual length of intragenic maps is about 1000 base pairs, corresponding to the structural gene and perhaps a few regulatory sequences such as promotors. This gives a rough estimate of the frequency of intragenic exchanges per kb, which can be compared with the average frequency of intergenic exchange calculated over the whole genome (Table 1, column 4). In lower eukaryotes (Ascomycetes), the frequencies of intragenic and intergenic exchanges are of the same order of magnitude. This is what would be expected on the hybrid DNA model for recombination[2-3-4]. In higher eukaryotes, the density of intragenic exchanges is comparable with what is found in fungi, but much higher than the density of intergenic exchanges calculated over the whole genome. That is, intragenic maps are of similar length in all organisms, whereas the frequency of crossover per unit length of DNA considerably decreases from lower to higher eukaryotes (Table 1, column 4). This is very striking for higher plants (*O. sativa*, *Z. mays*) where intralocus maps are about 500 times larger than expected from the extrapolation of crossover frequencies. The same probably applies to *D. melanogaster* although the data are more dispersed and, therefore, more difficult to interpret: Five loci are 10–50 times longer than expected from the extrapolation of crossover data, assuming an average gene to be 2 kb long (see also *albino* in spider mite). One locus (*ma–1*) is very short and would fit with a homogeneous distribution of intragenic versus intergenic exchanges. Two loci, (*garnet* and *rosy*, keeping in mind that *rosy* maps near the centromere in a region of unfrequent crossover) have intermediate values. On the whole, however, the data available for higher eukaryotes suggest a substantially greater frequency of recombination within, than between, the structural loci. If this is so, estimates of gene numbers in short regions of the genome such as the histo-incompatibility cluster of mammals should be revised, since the length of these regions (0.4 cM in mouse, see ref. 37) would be compatible with only a few genes.

Table 2 Frequency of spontaneous forward mutations per locus per gamete among eucaryotes (recalculated from papers quoted in ref. 12)

Organism	Screening system	Frequency of mutation/locus /gamete
S. pombe	Mutation to auxotrophy at five *ade* genes	1.6×10^{-6}
S. cerevisiae	Mutation to auxotrophy assuming 100 genes to lead to auxotrophic requirements	3.5×10^{-6}
D. melanogaster	Recessive lethals on chromosome I, II and III assuming 4,500 genes on these chromosomes	2.0×10^{-6}
M. musculus	Mutations at five coat colour loci	1.8×10^{-6}

A heterogeneous distribution of intragenic versus intergenic exchanges would have to be reconciled with the idea that both types of exchanges result from the formation of a hybrid DNA molecule[2,3]. One possibility is that, though hybrid DNA may occur with a roughly equal probability everywhere on the genome, strand isomerisation leading to crossover[2,3] is prevented by some features of the chromatine in higher eukaryotes. This would depress crossover without affecting non-reciprocal events. One would then expect, however, intragenic recombination to be predominantly associated with a parental distribution of flanking markers, which is not observed in the few cases analysed so far in *Z. mays*[14,15] or *D. melanogaster*[16,26].

I propose that this distribution of exchanges directly reflects the heterogeneous distribution of hybrid DNA on the chromosomes. That is, hybrid DNA formation may be mainly confined to the structural genes on the chromosomes of higher eukaryotes, in which case the frequency of exchanges per unit length of DNA should be much higher when calculated over short, intragenic distances than over the whole genome.

Cytological data show that the bulk of each chromosomal pair lies outside the synaptonemal complex and so cannot participate in recombination. It is not known whether this is randomly generated at each meiosis during the building up of the synaptonemal complex, or whether some mechanism restricts pairing always to the same stretches of the DNA, which may, for example, be regions with a dense distribution of structural genes. Alternatively, the necessity to keep the structural genes accessible to transcription may impose some basic constraint on chromatin organisation, resulting in them being preferentially accessible to hybrid DNA formation.

The fitness *ad equilibrium* of a polymorphic population is at a maximum in the absence of recombination[17], which may account for the linkage maps being roughly constant despite the increase in genome length. As for intragenic recombination, it results in the formation of new alleles and, provided a reasonably high degree of gene polymorphism is assumed, may substantially affect the speed of gene evolution. Unlike mutation, however, its rate might be easily modulated in a site-specific way, assuming that the specific *rec* genes of *N. crassa*[41] are of general occurrence in eukaryotes. It may then have been selected as advantageous by organisms placed in a variable environment.

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n-Butyrate causes histone modification in HeLa and Friend erythroleukaemia cells

LEDER and Leder[1] have reported that low concentrations of *n*-butyrate cause Friend erythroleukaemia cells to begin globin synthesis. Apparently *n*-butyrate can reverse that part of viral transformation which prevents the expression of differentiation in these cells. Prasad and Sinha[2] have summarised the effects of *n*-butyrate on neuroblastoma, HeLa, and other cell types. They and others[3–10] have seen reversible inhibition of proliferation, decrease of DNA content, morphological modifications, and increases in the production of specific enzymes, such as adenylate cyclase, alkaline phosphatase, and a sialyltransferase. The present paper describes rapid, dramatic, and reversible increases in histone acetylation in the presence of *n*-butyrate.

Nuclei from *n*-butyrate treated HeLa and Friend erythroleukaemia cells contain greatly increased amounts of modified forms of histone H4 (Figs 1, 2). Total histones from nuclei of cells after 24 h of treatment show several strong, slower moving H4 bands when examined in acid urea gels[11] and in Triton DF16-acid urea gels[12]. Their electrophoretic positions correspond to the mono-, di-, tri-, and tetra-acetylated H4 histones which occur during normal histone biosynthesis[13]. For this reason and because of the experiments described below, we believe the modified H4 histones to be acetylated. In control cells only a small amount of mono-acetyl-H4 is present, whereas this is the dominant component in treated cells. In HeLa cells the extent of modification, although marked at 1 mM, increases at higher *n*-butyrate concentrations (Fig. 1*a*). H4 acetylation and H3 modification in HeLa cells are already noticeable after only 1 h in 5 mM *n*-butyrate (Fig. 2), the earliest effect reported. Friend cells also show the acetylated-H4 pattern after 24 h in 1 mM *n*-butyrate; it becomes more pronounced at 3 mM (Fig. 1*b*).

In both HeLa and Friend erythroleukaemia cells the histone H4 pattern reverts to normal when cells treated with *n*-butyrate for 24 h are shifted back to control medium for a further 24 h (Fig. 1*c*, *d*).

In both cell lines there is also a clear alteration of the histone H3 band with an increase of slower moving material. This is a broadening of the H3 band rather than the appearance of new distinct bands. There is a striking increase in the relative amounts of H1 (Fig. 2). These changes are also reversible within 24 h after removal of *n*-butyrate.

To investigate the nature of the histone modification, total acid extracted histones from HeLa cells treated for 24 h with 5 mM *n*-butyrate were completely digested with trypsin and Pronase[14]. After separation from the proteolytic enzymes by Sephadex G25 column gel filtration, the free amino acids were analysed on a Durram analyser Model D500. A peak corresponding to ε-*N*-acetyl-lysine was found just in front of glycine in the position of a standard prepared from acetylated bovine serum albumin[15]. This peak was absent in the sample prepared from control cells. No evidence for ε-*N*-*n*-butyryl-lysine was found between alanine and valine in the position of an ε-*N*-*n*-butyryl-lysine standard. The amount of ε-*N*-acetyl-lysine was 1.7 mol per mol H4. It is not yet known whether these acetyl groups are all on H4 or whether they are more generally distributed. Quantitative analysis of the scans of gels shown in Fig. 2 indicated that the sum of the H4 components in *n*-butyrate treated cells was equal to the sum of the H4 components in control cells and that no H4 had been lost. The proportion of acetylated H4 reaches approximately 80% of total H4 at 24 h in 3 mM (Fig. 1*a*) or 5 mM *n*-butyrate (Fig. 2). In addition, we believe that the modification is

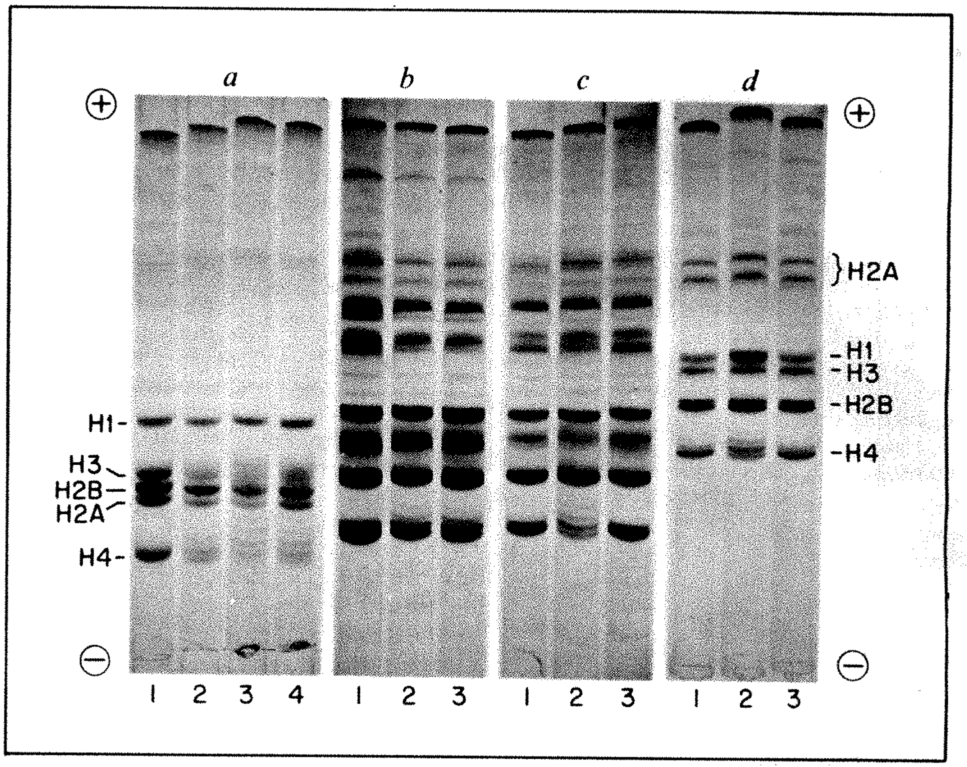

Fig. 1 Effect of *n*-butyrate on histone modification in HeLa and Friend cells. HeLa cells were maintained at 37 °C in suspension culture at 0.1–0.5 × 10^6 per ml in Joklik's modified minimum essential medium, containing 7% horse serum. Friend erythroleukaemia cells (clone 755-PC-4) (ref. 20) were grown at 37 °C in suspension in α medium[21] lacking nucleosides and supplemented with 15% foetal calf serum. They were maintained at 0.05–0.5 × 10^6 per ml to permit log-phase growth. Aliquots of a stock solution of 1 M Na *n*-butyrate in Ca^{2+}- and Mg^{2+}-free phosphate buffered saline (final *p*H 7.3) were added to cultures 24 h before histone extraction. Preparations of nuclei and histones were carried out at 0–4 °C. Nuclei were prepared from washed cells by Douncing and histones extracted[22] in 0.4 N H_2SO_4. *a*, Histones were precipitated with 10 volumes of acetone, dried and electrophoresed on acid–urea 15% acrylamide gels[11] at 20 °C for 5 h; the gels were prepared in 0.125% ammonium persulphate. *b*, *c* And *d*, histones prepared as before, were run on 15% gels containing 6 M urea and 0.38% Triton DF-16, according to the method of Alfageme *et al.*[12]. Staining was with 0.1% amido black in 40% methanol, 7% acetic acid. *a*, HeLa cells were either collected without *n*-butyrate treatment (gel 1) or after exposure to 1, 3, or 5 mM *n*-butyrate for 24 h (gels 2, 3, 4, respectively). *b*, Friend cells were collected without *n*-butyrate treatment (gel 1) or after exposure to 1 or 3 mM *n*-butyrate for 24 h (gels 2, 3, respectively). *c*, Untreated HeLa cells (gel 1), cells exposed to 5 mM *n*-butyrate for 24 h (gel 2), and 24-h *n*-butyrate-treated cells washed and resuspended in fresh medium without *n*-butyrate for 24 h (gel 3). *d*, Untreated Friend cells (gel 1), cells exposed to 1 mM *n*-butyrate for 24 h (gel 2) and 24-h *n*-butyrate-treated cells washed and resuspended in fresh medium without *n*-butyrate for 24 h (gel 3).

acetylation and not phosphorylation, since incubation of the modified histones with *Escherichia coli* alkaline phosphatase[16] leaves the modified H4 pattern intact.

We are particularly interested in the effect of *n*-butyrate on the proliferating malignant Friend erythroleukaemia cell and on the proliferating HeLa cell, which is also derived from a malignant cell. In the Friend cell differentiation is blocked. *n*-Butyrate induces the continuation of differentiation and the production of specific products, as well as the striking accumulation of acetylated histones. We do not know whether there is a causal relationship between histone acetylation and these observations. Darzynkiewicz *et al.*[17] and Nudel *et al.*[18] have reported subtle changes in the structure of Friend cell chromatin using either dimethyl sulphoxide (DMSO) or *n*-butyrate as inducing agents.

It is not known whether *n*-butyrate affects tumorigenicity of the Friend cell or whether it affects DNA synthesis. If it behaves like the original inducing agent, DMSO, we would predict a decrease in tumorigenicity and, by analogy with our HeLa cell experiments (Hagopian, M.G.R., Swartz and V.M.I., in preparation), an

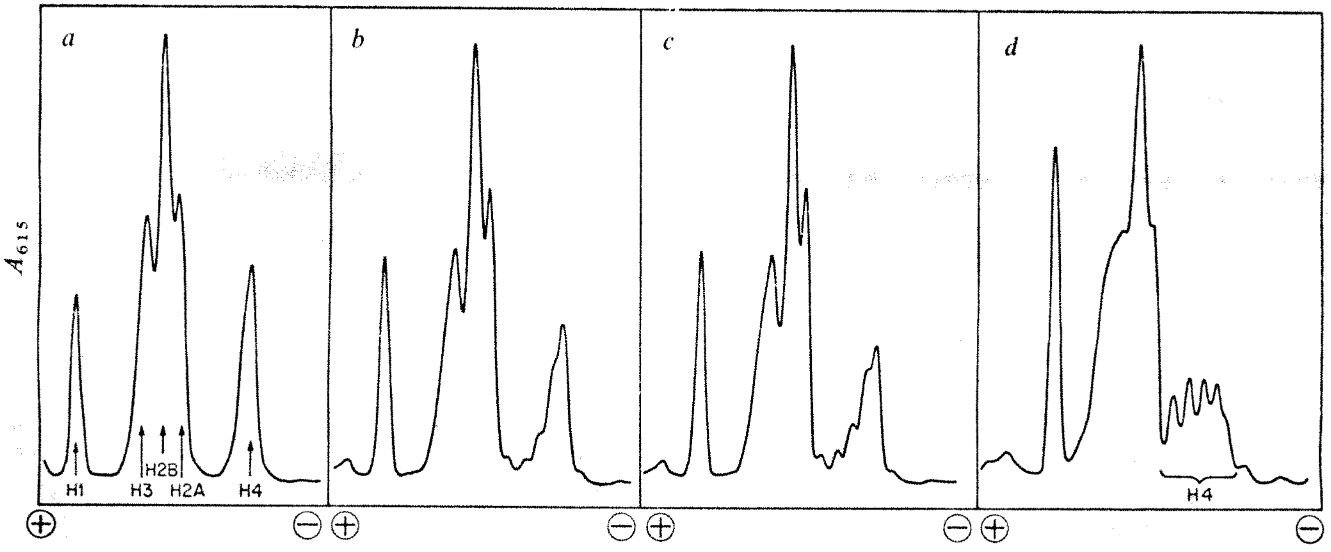

Fig. 2 Time course of *n*-butyrate effect on HeLa histones. Nuclei were prepared and histones extracted as described in Fig. 1. Cells had been exposed to 5 mM *n*-butyrate for *b*, 1 h; *c*, 2 h and *d*, 24 h. Samples were electrophoresed on acid urea gels and stained as in Fig. 1. The gels were scanned at 615 nm with the full scale absorbances adjusted to normalise peak H2B. *a*, Control.

inhibition of DNA synthesis. Moore and Chalkley[19] using cultured hepatoma cells suggest an interdependence of the level of histone H4 acetylation and the rate of cell division. We do not observe acetylation changes in the histone patterns of DMSO-treated Friend cells (J.R.N., unpublished); perhaps the two agents act at different points of these complex processes. Further experimental work is needed to look for a causal relationship between the accumulation of polyacetylated histones in the presence of *n*-butyrate on the one hand, and the inhibiting of DNA synthesis and the switching of malignant cells to non-malignant differentiating cells on the other.

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Yeast virus-like particles possess a capsid-associated single-stranded RNA polymerase

MANY species of fungi possess virus-like particles with double-stranded RNA (dsRNA) genomes[1,2]. In yeast the dsRNA genome has been shown to be the genetic determinant of the 'killer' character[3–10]. No cycle of lysis and reinfection has been demonstrated for 'viruses' of this group and replication is integrated with host growth[1,2]. Particles from several species of fungi have been shown to possess RNA polymerase activity and we have shown that yeast particles isolated from growing cells synthesise dsRNA (refs 11–14). We report here that particles isolated from non-growing cells synthesise both dsRNA and a single-stranded (ssRNA) species with properties suggesting that it is a complete transcript of the dsRNA genome. Our results with particles from growing cells suggested that the replicative cycle of yeast 'virus' resembles that of reovirus[14] and does not occur through the 'doubling cycle' which was put forward by Buck[13] to account for his results with *Penicillium stoloniferum* S. virus. The discovery of a ssRNA polymerase activity in yeast particles is consistent with the reovirus type of replicative cycle.

Particles were prepared from stationary phase cells of the strain 3/A1 (ref. 3) which possesses only P1 dsRNA and were incubated in the RNA polymerase reaction mixture as described in the legend to Fig. 1. The RNA reaction product was isolated and separated into ssRNA and dsRNA fractions by chromatography on cellulose[14,15]. These fractions were analysed by polyacrylamide gel electrophoresis (Fig. 1). Incorporation was found in both fractions but only 4%

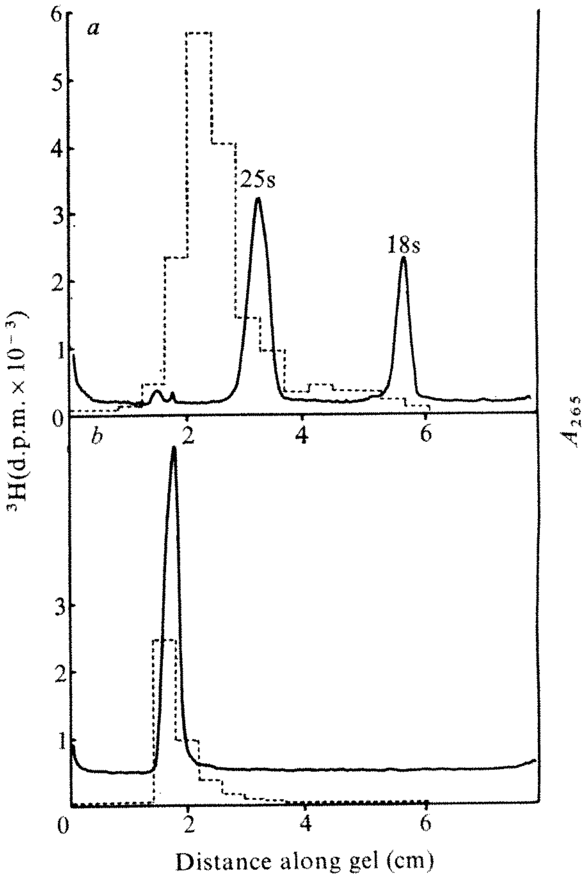

Fig. 1 Analysis of the ssRNA polymerase product on 2.6% polyacryladmide gels[27]. Yeast VLPs were prepared from strain 3/A1 after 10 d of culture by disruption of the cells with glass beads followed by polyethylene glycol precipitation of the VLPs (ref. 21) and isolation by differential and sucrose gradient centrifugation[1]. 2.9 A_{260} units of VLPs were incubated at 30 °C for 125 min in 0.7 ml of 0.05 M Tris-HCl buffer (*p*H 7.9) containing 0.15 M KCl; 0.1 mM EDTA; 3.0 mM $MgCl_2$; 0.20 mMGTP; 0.20 mM ATP; 0.20 mM CTP; 0.20 mM ^{3}H-UTP (330 mCi $mmol^{-1}$) and bentonite (2 mg ml^{-1})[13]. RNA was prepared by addition of 2 volumes of 0.05 M Tris-HCl containing 0.1 M NaCl and 2% tri-isopropylnapthalene sulphonate followed by two extractions with phenol and alcohol precipitation with 100 µg of yeast tRNA as carrier. CF-11 cellulose chromatography[15,16] was used to separate the ssRNA and dsRNA followed by reprecipitation and analysis on gels. Figure 1*a* shows the A_{265} (solid line) and radioactivity profiles (dotted line) of a gel loaded with 1/7th of the total ssRNA product from the reaction mixture described above plus 30 µg of total yeast nucleic acid. Figure 1*b* shows similar profiles for the total dsRNA loaded on to the gel after digestion with ribonuclease A (1.0 µg ml^{-1}) in 1×SSC (0.15 M NaCl, 0.015 M Na citrate, *p*H 7.0) for 10 min at 17 °C. The radioactivity profile was obtained by slicing the gel into 4-mm slices, digesting the slices with 0.5 ml H_2O_2 overnight at 60 °C and scintillation counting after addition of 5 ml of scintillation fluid (Nuclear Enterprises NE 250) to each vial.

of the counts were found in dsRNA. The major ssRNA product migrates as a single species with an estimated molecular weight of 1.6×10^6 relative to yeast ribosomal RNA standards[16]. This value is in good agreement with the estimates of the molecular weight of denatured P1 dsRNA obtained by polyacrylamide gel electrophoresis in formamide, which were 1.60 and 1.63×10^6, but is not consistent with our reported estimate of 2.5×10^6 for P1 dsRNA, estimated from aqueous gels[16]. The molecular weights of the *Aspergillus foetidus* S. virus dsRNA, however, which we used as standards have been re-estimated as 4.0×10^6 and 2.5×10^6 (personal communication from K. W. Buck and G. Ratti). This has enabled us to revise our molecular weight estimate for P1 dsRNA to 3.3×10^6. This figure agrees well with the formamide gel data.

The inclusion of bentonite in the reaction mixture is necessary to eliminate contaminating ribonuclease activity[17]; without bentonite the ssRNA product was found to be of low molecular weight. Because a low molecular weight product was found when VLPs from growing cells where incubated without bentonite[14] it is probable that they too possess ssRNA polymerase activity.

The gel profile of the dsRNA labelled in the reaction differs from that made by VLPs from growing cells in that it was of normal genome size and its mobility was unaltered by ribonuclease digestion (undigested sample not shown). This can be explained in three ways· it may be the result of the completion of short unreplicated ssRNA 'tails' on dsRNA molecules, as suggested for the dsRNA polymerase activity found associated with bacteriophage Φ6 virions[18]: it could be produced by a doubling reaction in which a small proportion of the ssRNA transcripts become templates for the dsRNA polymerase as is thought to occur in *P. stoloniferum* S. particles[13]: or it may be that the mechanism of the transcription reaction is not completely conservative as in reovirus[19] but semi-conservative with the ssRNA product being produced by strand displacement This latter mechanism is thought to operate *in vivo* during the replication of bacteriophage Φ6 (ref. 20). We are attempting to distinguish these alternative mechanisms experimentally.

The first report of a fungal virus polymerase producing ssRNA was from Ratti and Buck[21], who found that 70% of the *in vitro* product formed by particles of *Aspergillus foetidus* S. virus was sensitive to ribonuclease while the remainder was resistant and was assumed to be dsRNA. If the mechanism of the transcriptase reaction is strand displacement, the ratios of ds to ssRNA synthesised both in yeast and *Aspergillus foetidus* particles suggests that only in a few active particles which can initiate several rounds of synthesis does the reaction occur.

That the 1.6×10^6 molecular weight ssRNA product is a faithful transcript of the P1 dsRNA genome was substantiated by the hybridisation data shown in Fig. 2. The annealing conditions used, which are described in the legend to Fig 2, are based on those of Kielland-Brandt and Nilsson-Tillgren[22]. An excess of unlabelled P1 dsRNA was denatured and reannealed in the presence of ssRNA polymerase product. The renatured RNA was digested with ribonuclease and analysed on polyacrylamide gels. Fig 2*a* shows that the peak of activity and the peak of accurately renatured dsRNA coincide in the gel, that is, the ssRNA product is very near to half genome size as is the case with reovirus transcripts hybridised to genome dsRNA (ref. 23). The trailing shown by the peak of activity down the gel was also seen when the gel was stained although it is not very clear from the A_{265} profile. It is due to the heterogeneous population of dsRNA molecules which are generated by the digestion of network structures formed during the annealing[23]. Figure 2*b* shows a control sample which was not subjected to denaturation but was simply incubated at the annealing temperature. No activity is found on the gel which confirms the ribonuclease sensitivity expected of ssRNA and shows that it does not form ribonuclease resistant dsRNA by a process of self-annealing. The ssRNA product thus seems to be a transcript of a single strand of the dsRNA.

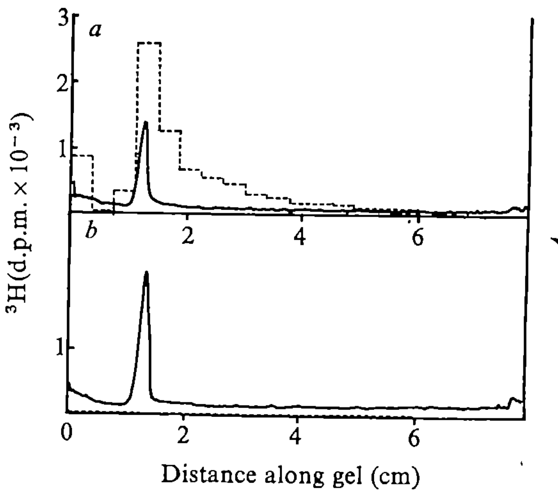

Fig. 2 Hybridisation of the ssRNA polymerase product. Polymerase product was taken up in 2.0 ml of 1×SSC containing 45% (v/v) formamide and 20 μg of unlabelled Pl dsRNA. The sample was divided equally, sample (*a*) was heated to 85 °C for 3 min and then incubated at 60 °C for 30 min, sample (*b*) was incubated at 60 °C for 33 min Both samples were precipitated with 2 vol. of ethanol after the addition of 100 μg of tRNA as carrier and loaded on to 2.6% polyacrylamide gels[27] after a 10 min digestion with ribonuclease as described for sample (*b*) in Fig. 1. Electrophoresis was for 6.5 h at 3.8 mA per tube. A_{265}, solid line; radioactivity, dotted line.

The amount of input ssRNA entering hybrid was calculated from the results of a separate experiment at 69%. This experiment included a sample which was neither denatured nor ribonuclease-digested. The degree of renaturation in this experiment, calculated by a comparison of the area of the dsRNA peaks in the A_{265} profiles, was found to be 65%.

The data together with our previous results show that both the polymerase activities required to undergo the asynchronous cycle of replication utilised by reovirus[19] are found in the yeast VLP. Bruenn and Keitz[24] have shown recently that yeast dsRNAs lack the 7-methyl guanosine 'cap' structures which are found both at the 5′ end of the + strands of reovirus dsRNAs[25] and in yeast mRNAs (ref. 26). They have suggested that this indicates that replication does not proceed by the reovirus cycle. We are investigating the *in vitro* methylation of the ssRNA polymerase product together with other aspects of its structure and synthesis.

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Enzymology in supercooled water

Recent years have witnessed an increasing interest in applications of low temperature techniques to studies of enzyme-catalysed reactions, the aims being the temporal resolution of the contributing reactions and the stabilisation of intermediate species. To overcome the low temperature limit set by the freezing point of the aqueous medium mixtures of water and various polar organic solvents have been used[1–4]. Although these aqueous mixtures affect the solvation interactions of the enzyme and its conformational stability[5], they can be shown to influence neither substrate specificity, nor the reaction pathway. We have investigated the feasibility of supercooled water to achieve conditions closer to the physiological ones. To circumvent freezing by heterogeneous nucleation, the aqueous phase containing enzyme, substrate and buffers was emulsified in an oil which was supersaturated with a water insoluble surfactant[6]. We here present the results on bacterial cytochrome P_{450}, a mono-oxygenase which has already been studied in mixed solvents[1,2]

Sorbitan tristearate was added to corn oil or safflower oil at 2.5% (w/v) and dissolved by gentle heating. An aliquot (1.5 ml) of an aqueous solution containing the enzyme in phosphate buffer, KCl and camphor (substrate) was emulsified in 3.5 ml of the oil phase at 10 °C. This was achieved by manual shaking, followed by microemulsification in a blender, until a droplet size of 1–5 μm diameter had been obtained. Optical spectra were recorded at temperatures down to −30 °C using an Aminco–Chance DW2 spectrophotometer. The various redox states of the substrate bound enzyme could be observed at low temperatures. The equilibrium constant governing the spin state equilibrium of the ferric cytochrome P_{405} was measured at several temperatures. The Van't Hoff ΔH at pH 7 agreed well with the corresponding value at ambient temperatures (10.3 kJ mol^{-1}) (ref. 7) but differed significantly from the low temperature value (30.1 kJ·mol^{-1}) determined in a 50% aqueous ethylene glycol solution[8].

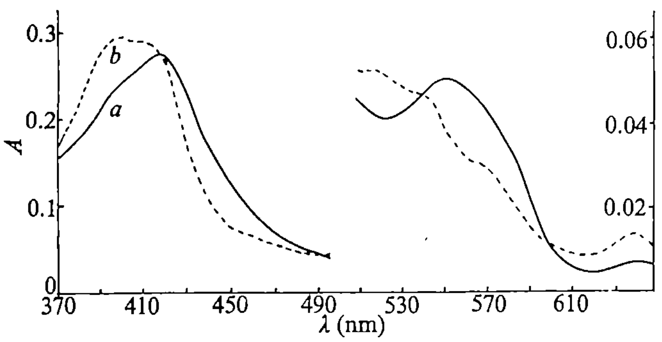

Fig. 1 Spectra of (*a*) oxy-ferrocytochrome P_{450} ($\mathrm{Fe_S^{2+}.O_2}$) stabilised at −20 °C in emulsified supercooled water, and (*b*) the same sample after warming and maintaining at 20 °C for 40 min and subsequently recooling to −20 °C. The long wavelength part of the spectrum (right-hand ordinate) was recorded with a higher sensitivity.

To investigate the possible stabilisation of labile species at subzero temperatures the unstable compound P_{450} ($\mathrm{Fe_S^{2+}.\ O_2}$) was formed at 0 °C, quickly emulsified and cooled to −20 °C in the spectrophotometer. The absorption spectrum in Fig. 1 clearly shows the maxima at 418 and 552 nm, characteristic of the oxy-ferrocytochrome. Repetitive spectra taken over a period of 1 h showed no evidence of autoxidation. After heating the emulsion to 25 °C for 20 min and recording another spectrum at −20 °C, the compound had been totally transformed to $\mathrm{Fe_S^{3+}}$. All spectra recorded in supercooled water were identical to those observed in normal conditions, except for a low temperature-induced sharpening of the peaks.

The preliminary experiments indicate that the extension of the accessible temperature range to the homogeneous nucleation temperature (approximately −40 °C) permits the stabilisation of enzyme-substrate complexes, and of labile intermediates and makes possible the temporal resolution of the enzyme catalysed reaction. A more detailed account of this work is in preparation.

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Corrigenda

In 'Lead isotope measurements from the oldest recognised Lewisian gneisses of north-west Scotland' by H. J. Chapman and S. Moorbath (*Nature* **268**, 41; 1977), line 4 in paragraph four should read . . . The isochron yields an age of 2680±60 Myr (2σ), which . . . .

In 'Eighty thousand β-adrenoreceptors in a single cell' by D. Atlas, F. Hanski, A. Levitski (*Nature*, **268**, 145; 1977) the ordinate label of Fig. 1*c* should read . . . ($\mathrm{M^{-1}\times10^{-14}}$).

In 'Histone content of germinating pea embryo chromatin decreases as DNA replicates' by F. Grellett, M. Delseny and Y. Guitton (*Nature* **267**, 724; 1977) the labelling of the peaks in Fig. 1 should read (from left to right): H1 (30,500; 26,500; 24,800), H2B (16,800), H2A (15,100), H3 (13,500), H4 (10,500).

reviews

A zoophile's testament

Eric Ashby

The Moral Status of Animals. By Stephen R. L. Clarke. Pp. 221. (Oxford University : Oxford and London, 1977.) £5.95.

In fairness to the author of this book, it must be said that he is a professional philosopher and this reviewer is not. And in fairness to this reviewer it must be said that the book is not addressed to philosophers but to the public at large. Moreover, it is not written to inform : it is written to persuade, with an uncompromising vehemence unusual among philosophers. So Dr Clark has to put up with the fact that I'm not competent to comment on the subtleties of his logic, just as I have to put up with Dr Clark's assertion (p183) "that no-one has any standing" in discussing the theme of his book "who has not taken the simple, minimal step of abandoning flesh-foods."

For that is the demand—and 'demand' is not too strong a word—which Dr Clark makes upon the reader. "I am a committed crank and zoophile, and my hope is to convert my audience." "Those who still eat flesh when they could do otherwise have no claim to be serious moralists." Dr Clark is fully aware of the consequences which would follow if his mission were to succeed. There would be a phasing out of all animal husbandry, from sheep and poultry, to fisheries and (presumably) shellfish beds. The sight of sheep placidly grazing is offensive to Dr Clark; they are the innocent and unaware "inmates of our Belsens" (p80). If this seems immoderate and emotive language from the pen of a philosopher, Dr Clark defends himself with the statement : "I am very much more interested in achieving a practical issue than in the details of abstract philosophy, and am willing to argue on almost any basis to achieve that end." But even this defence does not excuse such nonsense as the assertion (p188) that "flesh-eaters pretend that their victims delight to serve them."

Of course, abstinence from all flesh foods is only one of the demands which Dr Clark makes upon us. There must also be abstinence from all vivisection and from the use of animals to test drugs, on the ground that we must not do to animals anything which we would not legitimately do to human beings.

The book is written in a learned, not to say pedantic style, with copious references to authorities from Porphyry in the third century AD to Roszak in the twentieth century. Dr Clark professes himself to be a Christian but he confesses to finding little support for his thesis in the Bible and he is embarrassed by Christ's participation in the catching of fish. He admits that there is some sort of hierarchy among living creatures—to murder a sheep is "perhaps in less degree" serious than to murder a shepherd—and he is evasive about insects, although he admits with reluctance (p82) that insects killed on impact with a car windscreen weigh "less heavily than a deliberate killing."

There is an appendix with advice on vegetarian cooking.

This is a deeply disappointing book, for the issues which Dr Clark discusses are important. A critical examination of them would be timely because—contrary to numerous reckless assertions in Dr Clark's book—the common man *is* aware as never before of his kinship with the rest of nature. No-one who has seen nature films on TV believes (as Dr Clark implies on p37) that animals are stupid; no-one doubts that they suffer pain, can be apprehensive of danger, and that many kinds of animals are capable of affection, not only within their species but even toward man.

The widespread empathy between human beings and the rest of the natural world creates an interest in the rights of animals. A careful and sober enquiry into the consequences of this empathy would be welcome. Any such enquiry would have to take into account three considerations, none of which Dr Clark has faced squarely.

First, if as Dr Clark admits, the intensity of concern diminishes as one passes from mammals to vertebrate and from vertebrates to insects, where does it stop? And if not at insects, why not include plants? (Christopher Stone has already written an essay, far more persuasive than Dr Clark's, on the question whether trees should have standing in the law-courts of America.)

Secondly, Dr Clark admits that it's not our business to save the rabbit from the fox or the fly from the spider, but he does not explain why man, as an integral unit in nature, should abdicate from the place he has occupied in the food chain for millennia. Hamlet's "We fat all creatures else to fat us and we fat ourselves for maggots . . ." is a curt but accurate summary of man's place in the biosphere.

Finally, much of Dr Clark's case rests on the premise that man must not treat animals with less concern than he treats his fellow men. In fact man treats animals with more concern: we may kill domesticated animals in order to eat them; we don't torture them because they do not share our political or religious ideologies. The intraspecific behaviour of animals to one another is, by and large, more compassionate than our own intraspecific behaviour. ☐

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Cell surface in embryogenesis

Cell Surface Reviews. Volume 1: The Cell Surface in Animal Embryogenesis and Development. Edited by George Poste and Garth L. Nicholson. Pp. 766. (North-Holland: Amsterdam, New York and Oxford, 1977.) $89.95; Dfl.220.

This is the first in a new series of volumes designed to collate literature on cell membrane research. Three more volumes (Virus Infection and the Cell Surface, Dynamic Aspects of Cell Surface Organisation, Membrane Assembly and Turnover) are promised within the year. After this, volumes will be added to the series annually. This book deals with the more general aspects of development.

The chapter by Trinkaus is a scholarly survey of cell movement. Widely held misconceptions are exposed and corrected. Arnold's review on cytokinesis is concise and lucid.

Cell communication is discussed in four of the reviews. Sheridan provides circumstantial evidence for gap junctions as a structural basis for low resistance coupling of cells. Uncoupling of cells in development may prove to be an exciting clue to the mechanism of control. Aspects of differentiation relating to cell or tissue position are examined in a splendidly iconoclastical spirit (McMahon and West) in both regulating and non-regulating systems. Most of the important research dealing with embryonic induction has been compiled by Saxén *et al.* Cell surface specificity in cell systems *in vitro* seems to be present (Maslow), but its relevance to actual developmental events can always be questioned.

Five of the reviews deal with the development of the mammalian conceptus and placenta. They might have been more usefully placed in one of the later volumes. Gwatkin restricts his consideration of the organisation of the cell surface of gametes to the mouse. This seems a pity since much of the interesting work in this field lies in invertebrate studies. Sherman and Wudl make provocative comments about the putative role of the blastocyst in implantation. The transport of molecules across placental membranes is lengthily listed (Miller, Koszalka and Brent) and some studies of cell surface antigens in mammalian developments are reviewed by Edidin.

The development in only three organ systems is covered, and in each a somewhat pedestrian note is struck. Hot or controversial issues have not been stirred up in these reviews about the heart (Manasek), limb bud (Ede), or immune system (Goldschneider and Barton).

All in all, the editors and reviewers have done a fair job. The publishers, however, can be severely criticised. The book costs ninety dollars, has many typing errors and is inadequately indexed. I have recommended this book to our library, but they cannot afford it.

Joan Feldman

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Solar planets

The Solar Planets. By V. A. Firsoff. Pp. 184. (David and Charles: Newton Abbot, London and Vancouver; Crane, Russak: New York, 1977.) £5.50.

CRUDELY one can divide science books into four categories, elementary and advanced, good and bad. Firsoff's book is elementary and bad.

There is nothing wrong of course with a book being elementary. This one is written for the amateur astronomer and purports to be a study of the Solar System written in the light of the wealth of new knowledge gleaned from the unmanned space probes which have recently visited Mercury, Venus, Moon, Mars, Jupiter and Saturn. True, the book contains plenty of details about these missions, seventeen black and white plates and no formulae. And obviously the book is not all wrong, in fact a good 95% is right, but the unsuspecting reader has no way of picking out right from wrong, fact from the authors' flight of fantasy, the solid ground from the castles in Spain.

We are told: "it seems that the Moon began its existence as an independent planet and was later captured by the Earth", "a limestone meteorite fell in 1926 at Bleckenstad and is said to have contained fossils", "Pluto may possibly belong to an older solar system or else be an immigrant from interstellar space"; "degeneracy is a condition derived from equations based on various simplifications which are always suspect". "I am not too sure that even in the Solar System ours is the only technical civilisation", "geography is fast becoming only a division of astronomy"; "fissile radioactive materials could become concentrated by geological processes into a natural atomic pile, which could occasionally blow up", and a host of other *non sequiturs.* Many facts pop out at us like rabbits from a hat accompanied by a similar aura of surprise and lack of explanation. The book also uses jargon freely. The reader, having no glossary, has to turn to other works for definitions of such terms as Hohmann transfers, coriolis force, grey-sphere radiative equilibrium, body tides, bond albedo and so on. Also in evidence is Firsoff's dislike of professional scientists. For example, "the idea of an inert Moon is still widely held among scientific officialdom, often in the teeth of overwhelming evidence against it". In discussing a dark pattern seen on one of the rocks on Venus pictured by Venera 9 Firsoff puts forward the idea that this could be a lichen growth—a genus from a high temperature biology based on sulphuric acid as the solvent: "of course this is speculation" Firsoff writes, "and speculation is a dirty word in professional circles—but many of the hypotheses that have been given an official accolade are even less well founded".

This book gives an entirely wrong impression of present knowledge of the Solar System and of the state of mind of the scientific enquirer who is presently investigating the planets. Not only do I advise people not to buy this book, I will go further, don't even bother to open it.

David W. Hughes

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Pursuit rotor performance

Remininiscence, Motivation and Personality. A Case study in Experimental Psychology. By H. J. Eysenck and C. D. Frith. Pp. xxi+430. (Plenum: New York and London; 1977.) $33.

THIS self-proclaimed "case study in experimental psychology" is concerned exclusively with the phenomenon of reminiscence as observed in studies of pursuit rotor performance. The pursuit rotor is a piece of apparatus which requires a human subject to keep a stylus in contact with a small target on a rotating turntable. The reminiscence effect refers to a gain in performance—and possibly in learning—when a rest period is introduced after a period of practice. The authors' introduction asserts that "whether the accumulated work done by generations of students on the problems discussed in this book has been worth the trouble must be left to the reader to judge." The authors are presumably optimistic about the reader's verdict; but not, I think, with good reason.

The major virtue of the pursuit rotor is said to be the replicability of the data which it generates—"perhaps the most reliable set of observations in experimental psychology." The pursuit rotor, we are told, is "the psychologists' favorite piece of apparatus." These claims seem absurdly parochial, but even if taken seriously they are offset by the authors' remark—in criticising a study which disagrees with their views—that "the literature on reminiscence is especially rich in 'failures to replicate.' "

The book does, however, provide a detailed history of research on this narrow problem. The same studies which the authors had earlier interpreted in terms of "inhibition" theory are now interpreted in terms of "consolidation" theory. The fit of the new theory to data, like that of the old, is not so striking as to compel belief. The theoretical concepts used have not arisen from the study of reminiscence in the pursuit rotor task, but have been imported from general psychology. There is no shortage of available 'explanatory' concepts.

This work will be of interest, and possibly of value, to those who are concerned with the pursuit rotor and related matters. There are, however, many experimental psychologists who would have preferred a more fertile subject for such extensive case study.

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Regge theory

An Introduction to Regge Theory and High Energy Physics. By P. D. B. Collins Pp. xiii+445. (Cambridge University: Cambridge, London and New York, 1977.) £29.

OVER the past fifteen years, the theory and phenomenology of high energy collisions of strongly interacting particles have developed into a powerful and coherent picture The basic idea is a subtle generalisation of Yukawa's idea of forces being due to exchanges of particles. This idea is, of course, built on the classic example of electrodynamics, where exchange of photons provides the electromagnetic forces between electrons. When this idea is applied to the exchange of known strongly interacting particles of high spin it leads to absurdities in specific kinematic regions. The classic mathematics of Regge and its applications by Chew, Frautschi, Mandelstam and others was that an object called a Regge pole could be usefully defined, which subtly changed from being one particle into another for different kinematics. Thus, exchange of a Regge pole is equivalent to exchange of many Yukawa particles in a simple way. The Regge pole also clearly serves to classify the several (many?) particles whose exchange it controls. These particles all have different spins. Thus, it provides a classification of a totally different nature to the canonical isospin, strangeness (SU(2) and SU(3)), beloved of group theorists, which classifies objects of the same spin.

This theory has been developed and tested in a wide variety of collision processes and has encountered no conspicuous disasters in confronting the data. The status of experiment and theory in this field are superbly reviewed by Dr Collins in this elegant book. The level is beautifully judged to lead a new research student into the depths of this field and start him on his research career. On the other hand, as a worker in this field, it gave me great pleasure to see the material laid out so coherently. The book contains a judicious amount of data without which one can so easily become lost in the theoretical intracacies.

After a quick introductory review of the basic assumptions of scattering theory, Dr Collins turns to the fundamental derivations of the complex angular momentum plane and models which show the existence of Regge poles. The basic Regge trajectories are then discussed and the connection with internal symmetry groups brought out in detail. These trajectories are found from fitting the data. The details of these fits are admirably described.

Because of analyticity, if we know the high energy behaviour of scattering amplitudes, strong constraints are imposed by Cauchy's theorem on the low energy amplitudes. These constraints are typically given by the so-called finite energy sum rules and duality. These are again succinctly described by Dr Collins.

In a relativistic theory it is known that as well as the Regge poles to be exchanged there are so-called 'Regge cuts' which come from exchange of two Regge poles. These correspond to re-scattering cross-sections in the manner of Glauber theory. This is an area of great theoretical interest in view of the rising total cross-sections found at the CERN intersecting storage rings using the highest available energies This chapter is followed by a discussion of the ingenious way in which Regge poles contribute to collisions with three or more particles in the final state. In particular, the application to inclusive processes where, say, two protons collide to turn into a pion plus an unknown number of other final state particles, is described in detail. This is a true success of the theory in a fundamentally relativistic situation, with many particles being produced out of pure energy.

Finally, the relatively unsuccessful attempts to calculate the Regge trajectories from more fundamental theories are described. This joins naturally to the last chapter which connects with conventional field theories and their Regge trajectories. A brief discussion of deep inelastic electron–proton scattering is given, with its hint that there may be point constituents in protons, unlike the fuzzy picture suggested by Regge theory.

In conclusion, this is a superb introduction to strong interaction dynamics with an admirable blend of theory, speculation and experimental data which I cannot recommend too warmly.

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Mercurial chemistry

The Chemistry of Mercury. Edited by C. A. McAuliffe Pp. 288 (Macmillan: London, 1977.) £25.

WHILE reading this slim volume one is conscious of the fact that each page costs just under 10p and it is thus rather annoying to find that 29 pages are completely blank, contain only titles or are less than half full.

Unhappily the book falls far short of its title; certainly it is not a treatise on mercury as claimed in the Introduction. There is no attempt made to compare mercury with its congenors or with the the alkaline earth elements, and the aqueous chemistry of Hg^{2+} is dismissed in the eight lines of "chapter" 17. I had hoped that one could have picked up this book and had the whole of mercury chemistry at one's finger tips but, since the style of the main chapters is somewhat similar to that of the *Annual Reports of the Chemical Society,* access to a good library is essential to obtain sufficient background knowledge. Although "to avoid duplication, work presented in recent reviews is not duplicated here", there is considerable overlap in Chapters 11 through 17 with Professor Aylett's article (about 1969) in Pergamon's *Comprehensive Inorganic Chemistry*—indeed, in several places the two descriptions of Hg_2^{2+} and Hg^{2+} chemistry, necessarily, read almost word for word; naturally this book contains a little more up-to-date material (May, 1975). I looked for, but did not notice, any mention of Aylett's review among the copious references.

The first part of the book gives a readable account by Farrar and Williams of the history of mercury, but takes up 45 precious pages; numerous quotations from translations of Pliny and alchemical texts are presented. Part two by Levason and McAuliffe discusses the coordination chemistry of Hg_2^{2+} and Hg^{2+}. The organic chemistry of Hg (II) is dealt with in considerable detail by Bloodworth in Part three, and the biochemistry and toxicology of mercury (Falchuk, Goldwater and Vallee) are dismissed in a disappointingly brief section considering their current intense interest.

There would seem to be no justification for a short book of this title, and certainly the price will keep it out of the reach of all but the most dedicated of mercury chemists and the well-endowed libraries. On reading the title I had high hopes as to the contents but these hopes were largely shattered as it became clear that the book was meant as a review and not a readable and critical account of this most unique element. Approximately two dozen actual printing errors were noticed, whereas broken type occurred with too high a regularity for a book in this price range.

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Structure and properties of the solid surface

The Chemical Physics of Surfaces. By S. Roy Morrison. Pp. xii+415. (Plenum: New York and London, 1977.) $47.40.

SURFACE STUDIES constitute a substantial growth area in contemporary scientific research. During the past decade or so, a host of new techniques have provided insights into the structure and properties of the solid surface. These developments in turn have had a bearing on those technologically important processes where surface interactions are important—in solid-state devices, in oxidation, in corrosion and in catalysis.

In the past four years, several small monographs have appeared on the structure of the solid surface (Oudar, Blakely, Samorjai, Prutton); in those cases where reactions have been considered, however, they have been treated largely in terms of the atomistic nature of the surface. The present book attempts to re-dress the balance by emphasising the electronic band structure of the surface and the role of surface states in determining the interaction of the surface with a gaseous or liquid environment. The book is thus aimed primarily at those interested in surface chemistry and tends to deal more with semiconductors than with other types of solid.

The author expresses the hope that the book will also appeal to the mature surface physicists but it seems to me that it is too specialised and that it lacks more figures of the type given in Figures 5.1 and 5.2, where the lobes of orbitals and so on are of particular assistance to the non-chemist, and a few more energy diagrams illustrating the electron or hole switching mechanisms would clarify some of the verbal accounts of surface interactions.

Again, the references (almost 1,000) cover mainly the period from 1970 to 1975. The book is thus essentially a concentrated review of the more important papers published over a relatively short period. As such, it is indeed a tribute to the author's encyclopaedic knowledge and understanding of recent work involving general aspects of surface interactions, especially in relation to semiconductor surfaces. It does, however, carry with it certain limitations. For example, because of Dr Morrison's overwhelming interest in catalytic processes and in the chemical properties of ionic and semiconducting solids, his account of surface interactions gives only passing reference to faceting and little more to the role of surface defects.

One of the most exciting (and perhaps depressing) features of surface science is the rate at which new techniques are continuously emerging. Dr Morrison's book deals with papers up to the end of 1975 and quotes ultraviolet photoelectron spectroscopy (UPS) as "the most important tool of the surface spectroscopies in providing us with information on surface states and adsorbate bonding". Since then, high resolution energy loss spectroscopy (HRELS) at extremely small electron energies has appeared on the scene and has shown that it is possible to detect the vibrational characteristics of adsorbed atoms or molecules. It thus promises to provide more direct information than UPS about the location and bonding of surface species. No doubt in two years' time it will be superseded by yet another technique. This is not, of course, a criticism of Dr Morrison's monograph. His book is a treasury of information and an essential acquisition for any laboratory engaged in surface studies involving catalysis and solid–gas or solid–liquid interactions, especially of semiconducting solids.

David Tabor

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Broad geophysical canvas

Our Changing Planet. By John Gribbin. Pp. 165. (Wildwood House: London, 1977.) £5.95.

THIS small book is intended to persuade laymen of the importance of geophysical research to Man's present needs, and also of its intrinsic interest. It ranges over a broad canvas, including the origins of the Solar System, oil, gas and metals, the possible relationship between sunspot activity, climate, biological evolution, and earthquakes, as well as the inevitable plate tectonics. This hotchpotch hangs together moderately well and avoids the doomwatch approach which often characterises such publications. The book is also well produced, with a pleasant appearance, a short layman's bibliography and an index. To complet the credit score, the number of printer's errors are few and these are amusing rather than annoying—larval plains on Mars, for example.

After commenting favourably on its readability, however, the credits become largely exhausted. The glib tone tends to be combined with an inadequate presentation, occasional misrepresentation and even straight errors. The importance of continental shelves as potential oil fields is clear enough without claiming that the Siberian Artic continental shelf is larger than Africa, and the English Channel does not immediately jump to mind as exemplifying the characteristics of the shallow seas in which oil deposits are laid down. Greek ideas on the cause of earthquakes, dismissed as silly, still seem appropriate in particular conditions, such as those at Agadir. Some arguments are seriously neglected, such as the isotopic information for the origin of the metal brines of the Red Sea, and others seem almost topsy-turvy.

Earth expansionists use the 'improved' fit of the continents on a smaller globe as part of their evidence in favour of expansion yet Gribbin sees the fit as a clear argument against. Although this reviewer's feelings about expansion make him side with Gribbin on this, the lack of evidence for expansion on the Moon, Mars, Venus, and so on, are the more telling arguments and are ignored.

It is also somewhat distressing to find, apparently, how provincial geologists are, compared with geophysicists. Apparently, geologists are concerned only with the status quo, and it is geophysicists who are concerned with the changing Earth. Although such cynical comments can be made only too easily, the most serious drawbacks for the lay reader, however, must be the lack of adequate illustrations and of clear exposition as to what, for example, plate tectonics is. If the initial picture had been presented more clearly, then one could almost, but not quite, forgive some explanations—for instance, how can North America override a spreading ridge? Simply because the Atlantic is spreading westward faster than the Pacific is spreading eastwards! In many cases, the glibness overtakes the information which is necessary, even for the lay reader. The Chandler Wobble, for example, is described as the pole dancing around, in stately fashion, relative to the stars, but the uninitiated will want to know the scale of this dance—is it confined to a barn, a ballroom or a continent?

I am sorry that I cannot recommend this book to anyone, which is not merely unfortunate but depressing. If John Gribbin had spent much longer on its preparation and confined himself to only two or three topics, it could have been excellent—but he didn't.

D. H. Tarling

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obituary

Sir Landsborough Thomson

Bassano Studio

ARTHUR LANDSBOROUGH THOMSON died on 9 June 1977 at the age of 86. With his death we have lost the last of that small group of men who, in the early years of this century, brought into being the organisation in this country for the national support of scientific research. A noted biologist, with a natural talent for administration, he combined in his own person the qualities required for translating scientific policy into administrative practice. It was, therefore, fortunate that circumstances brought him, at an early stage in his career, into the position of chief of staff at the headquarters of the then embryonic Medical Research Council.

Born in Edinburgh in 1890, Thomson spent his formative years in Aberdeen where his father was Regius Professor of Natural History. After graduating as MA in 1911, he elected for an academic career in zoology. In preparation for this, it was decided, in line with the spacious traditions of those days, that he should first broaden his education. So he went to study, first at Heidelberg, and then in Vienna from where he only just succeeded in escaping before the outbreak of the First World War.

Arrived back in Scotland, he immediately joined the Argyle and Sutherland Highlanders. With that distinguished regiment he went to France and saw hard service. It was then that his flair for administration first revealed itself. As a result he was recruited to the staff and ended the war, in the rank of Lieutenant-Colonel, as an Assistant Quartermaster-General at General Headquarters. He thus came to take a different view of his future. Returned to civilian life, he went to the Treasury. But he was only there for a short while. Sir Walter Fletcher, the then Secretary of the Medical Research Council, was looking for a man to build up the administrative side of his organisation; and Thomson was the obvious choice for the post.

When Thomson joined the MRC the entire staff at headquarters, apart from the Secretary, numbered no more than half-a-dozen persons. When he left, some forty years later, the figure was 130. Nevertheless, even then, the staff was remarkably small when set against the size of the Council's organisation and the scope of its activities. In part this was due to its having grown naturally rather than as a result of reforms imposed from outside. More especially, however, it was due to Thomson's wide range of capabilities. With his scientific background, and his continued personal involvement in research in ornithology, he was never in danger of mistaking means for ends and complicating administrative procedures accordingly. For him the first task was to identify the scientific requirements of any given problem and then devise measures for meeting these. Not that he ever fell into the opposite error of ignoring those basic principles and methods that are applicable to all administration, whatever its purpose. But he required that every case be approached on its merits, never on the basis of precedent. That was the atmosphere that permeated the whole office under his sway. As a result, agreements were reached with the minimum of fuss and, although prospective applicants might go away disappointed, they could seldom, if ever, feel they had been misunderstood.

But Thomson was far more than the head of the Council's machinery for supporting its purely research interests. Having a realistic appreciation of the social and political context in which the Council operated, and a shrewd eye for impending developments, he brought his own expertise to bear on the formation of policies for translating scientific knowledge into practice. An example is the part he played in creating the Public Health Laboratory Service. Originally conceived, in the mid 1930s, as a defence against bacterial warfare, this quietly came into being on the outbreak of the Second World War. Such things do not happen by accident. They are the fruit of realistic vision and informed understanding by those responsible for planning the logistics of such an operation. In the event, the threat this particular service was devised to counter never materialised, but the network of laboratories it provided proved so useful in extending our control over the ordinary infections of civilian life that it was put on a permanent footing and eventually transferred, as a going concern, from the Council to the National Health Service. That Thomson should have been accepted without question as chairman of the expert board which directed such a service, is a sufficient indication of how he was regarded by the scientific community. When I want the MRC, I was exceptionally fortunate in having him as a colleague and mentor, for to work with him was a professional education in itself.

Yet all the time he had another life: that of a distinguished ornithologist. From his earliest years, he had taken an informed interest in wild life. Later, this developed into his classical studies on bird migration. But, at heart, he was always a field naturalist and, in this context, his knowledge was encyclopaedic. Consequently, he was, at one time or other, chairman of all the major organisations for ornithology in Great Britain and, in 1954, President of the XIth International Congress held in Basle. After his retirement from the MRC, his interests in this field could develop freely. He travelled widely, especially in the tropics. He took a major part in the enquiry into the Serengeti Park. From 1954 to 1960, he was President of the Zoological Society of London, from 1964 to 1969, Chairman of the Council for Nature, and from 1967 to 1969, Chairman of the Trustees of the British Museum (Natural History). And, with the help of his devoted wife, he edited his great *New Dictionary of Birds*.

In spite of these commitments, when he came to retire, Thomson readily accepted an invitation from the Medical Research Council to write an account of its development. Having been personally involved in this for most of the period which it covered, he was in a unique position to undertake this task. The outcome, *Half a Century of Medical Research*, is far more than an institutional history. In it he sets out, with characteristic objectivity, the various developments in the British organisation of medical research and the administrative lessons to be learned from these. As such its relevance extends beyond the sphere of its immediate subject to a central problem of the scientific and technological age in which we are now living; namely, that of providing, not only for the development of scientific knowledge, but also for its integration into the machinery of government.

Like his old schoolfellow, Wilson Jameson, the architect of the National Health Service, Thomson understood that for any human institution to be a success, it must satisfy both of two fundamental requirements. It must be in conformity with the realities of that it seeks to organise. It must equally be in conformity with the deeply held sentiments of those who have to make it work. In his own time, Landsborough Thomson contributed his quota to realising these conditions and, thereby, to creating the institutions required if activities that depend on acts of individual judgment are to be integrated into the structure of social organisation. That was the deeper significance of his life's work and his essential legacy to future generations.

Harold Himsworth

Meirion Thomas

Meirion Thomas who died in his eighty-third year on 5 April 1977 at Tywyn in his native Wales will be remembered not only for his academic and athletic prowess but also for sterling personal attributes.

Born in North Wales in 1894, his early academic career as a student of University College of North Wales was interrupted when he was commissioned in the South Wales Borderers in 1914 and later transferred to the Special Companies Division of the Royal Engineers. On demobilisation in 1919 he went to Cambridge (Trinity Hall) where, inspired by his contacts with Gowland Hopkins, F. F. Blackman and Muriel Onslow, among others, he developed interests in plant physiology and biochemistry. He joined the Botany Department in the Newcastle division of Durham University as a lecturer in 1924, became Reader in Plant Physiology in 1942, and in 1946 Professor and Head of the Department where he remained until retirement in 1961.

His book *Plant Physiology,* first published in 1935, brought him to the fore because of its new approach to the subject with its emphasis on the experimental study of plant metabolism. It was reprinted and revised periodically to a fifth edition in 1973, always retaining his aim of educating students in the development of ideas and investigation rather than simply presenting the present state of knowledge. His main research interests as covered by his own publications embraced various aspects of respiratory and acid metabolism with occasional ventures into other fields e.g. melanism, but the sum total of his contributions to science and life could only be assessed by perusal of the long list of students, colleagues and friends who would readily attest that whatever they have achieved, and some have achieved great distinction, they owe much to their contacts at some stage with him.

Meirion Thomas's eminence in science was marked by his election to the Royal Society in 1949. Two other distinctions which gave him great pleasure, and of which he was justifiably proud, were the award of the Charles Reed Barnes Honorary Life-Membership of the American Society of Plant Physiologists in 1963, and the Honorary Degree of D.Sc. of the University of Wales in 1964.

At Cambridge he won a soccer blue and played cricket, being elected to the Crusaders Club in 1923. He continued enthusiastic participation in both with Northern clubs for many years and remained actively interested in them throughout his life. A few years after retirement, his love of Wales, its language, and its cultures drew him to Tywyn, but not as a recluse. He maintained contacts with all his friends, continued to attend scientific meetings and enjoy a full life. He will be remembered with respect and great affection by all who knew him.

S. L. Ranson

A. V. Nikolaev

Academician Anatolii Vasil'evich Nikolaev, a leading Soviet expert in geochemistry and the utilisation of natural resources died on February 13, 1977.

Nikolaev was born in 1902. He graduated from the University of Leningrad in 1924, and soon afterwards became involved in the problems of resource utilisation. From 1927 to 1931 he was head of the Pavlodar Salt Expedition of the Commission to Study Production Resources of the Academy of Sciences of the USSR, and from 1931 to 1935 he headed a similar, combined, expedition to the Kulunda area. Between 1934 and 1957 he worked at the Institute of General and Inorganic Chemistry of the Academy of Sciences in the USSR, and, simultaneously, held various teaching posts in Moscow, at the Polygraphic Institute and the Institute of Non-Ferrous Metals and Gold, becoming a Professor at the latter Institute in 1946. In 1957 he moved to Novosibirsk, to become Director of the Institute of Inorganic Chemistry of the Siberian Branch of the Academy of Sciences of the USSR. The following year he was elected a Corresponding-Member of the Academy of Sciences and a Praesidium Member of the Siberian Branch. He became a full member of the Academy in 1966.

Nikolaev's main works deal with the physical and chemical analysis of salt systems, thermography and radiochemistry. His interests were wide and his published titles reflect this diversity, ranging from *The Kulunda Salt Lakes and means of desalinating them* and *The physico-chemical study of natural resources,* to *Protection against radioactivity,* and *A study of the processes of oxidation of complex compounds of divalent platinum.*

In 1947, he won the Vernadskii prize, and, in 1967, on the occasion of his 65th birthday, he was awarded the Order of Lenin.

Vera Rich

C. W. F. McClare

Colin W. F. McClare, lecturer in biophysics at King's College London, died on 4 January 1977 at the age of 39.

He won an open Exhibition to Emmanuel College Cambridge from Felsted School in 1955 and read Natural Sciences, specialising in chemistry. His interest in applying chemical concepts to biological problems was already apparent in his PhD project on free radicals in biology and was developed further when as a Beit Fellow he studied energy transfer in nucleic acids.

After his appointment at King's College in 1963, he turned his attention to fundamental problems in bioenergetics, especially muscle contraction. He stressed the importance of time scales in thermodynamics and proposed a muscle model employing stimulated resonant energy transfer. He strongly advocated that the secret of ATP lies in the molecular details of its hydrolysis and not simply in its overall free energy change.

Colin was a person of great enthusiasm and intensity of thought. His interests ranged from practical methods of analytical biochemistry to the social and philosophical implications of science. His search for excellence and his competitive spirit showed in his love of discussion and in his sporting activities. As a teacher, he inspired his students through an outstanding ability to make ideas come to life.

He is survived by his wife Gill. His stimulating and ever-helpful presence will be missed by his many friends.

W. R. Lieb
I. Gonda

newly on the market

These descriptions are prepared by the staff of *Nature* on the basis of material provided by manufacturers. The Reader Enquiry Coupon faces the inside back cover.

Photoresist illuminators. Oriel. The high efficiency 1,000 W Photoresist illuminators produce highly intense uniform, collimated beams rich in the longwave ultraviolet and shortwave visible, the sensitive wavelength region for most photoresists. The unique optical system provides very high collection efficiency and excellent uniformity with an extremely simple alignment procedure.
Circle No. 42 on Reader Enquiry Coupon

Tube rotator mixer. Gallenkamp. A new tube rotator/mixer suitable for both vertical mixing and horizontal tube rotation, the drum accepts two or four plates of clips. Rotation speed is variable between 6 and 60 rev. min^{-1}, and a clutch on the drive motor allows thed rum to be rotated by hand for easy loading and unloading. A built-in timer enables runs of up to 60 min to be terminated automatically. The overall dimensions are 430×240×340 mm high.
Circle No. 43 on Reader Enquiry Coupon

***p*H meters.** Gallenkamp. Models 7 and 12 have large-scale meter readouts and are intended for routine and research operation respectively. Both read 0–14 (±0.05)*p*H and 1400–0–1400 (±14)mV. In addition, model 12 has an expanded scale which gives a full scale deflection for one *p*H unit at any value within the overall range or 100 mV range. Two additional, logarithmic, scales are provided for ion activity measurements. Models 109 and 113 have digital readouts. The former is a general purpose meter reading 0–14 (±0.01) *p*H and 1900–0–1900 mV. Model 113 also covers the full *p*H range and is accurate to ±0.005 *p*H unit, for research applications. Additionally it reads 1799–0–1799 mV and has an expanded scale facility. For routine laboratory and field studies, a battery-operated *p*H meter (Model 13) is available. This reads 2–12 *p*H with a 2 *p*H overrange at both ends of the scale, and 300–0–300 mV. Models 7, 12 and 113 are suitable for Karl Fischer titrations and all the *p*H meters are supplied complete with combination electrode.
Circle No. 44 on Reader Enquiry Coupon

Automatic tissue dehydrator. Reichert-Jung UK. The EM Tissue Dehydrator completely relieves the technician of tissue preparation for electron microscopy. Also, the process is made completely safe—dehydration taking place in a fully enclosed chamber—and highly consistent results are ensured. Specimens are accommodated in up to 51 porous capsules and the dehydration cycle is achieved in 9 automatically controlled stages. Timing of each stage is adjustable to ensure conformity with existing procedures, the tissue being finally taken up to 95% alcohol. There is also provision for immersion in 100% alcohol and propylene oxide, under manual control but still within the enclosed specimen chamber. At each stage, alcohol is added at a predetermined rate, the volume being adjusted to keep overall volume constant throughout the process. The process may be started or stopped at any stage and an alarm, incorporating a variable delay of up to 1 h, indicates cycle completion. On starting, a 50 s delay ensures that the dehydrator is purged of all undesirable vapours before operation.
Circle No. 45 on Reader Enquiry Coupon

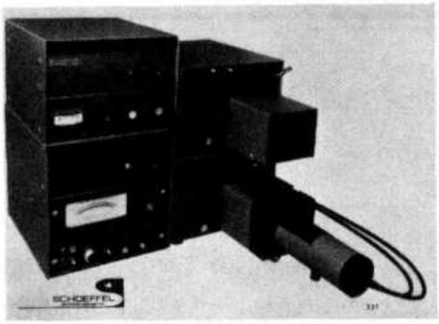
Reichert-Jung automatic tissue dehydrator.

Fume cupboard. Morgan & Grundy. Traditional fume cupboards contain many joints and corners. To overcome the problems this can cause, Morgan & Grundy have designed a new one piece fume cupboard liner. All corners are welded with a large radius and the base is an integral part of the fume cupboard, so there are no corners or joints. This new design is of stainless steel, making it ideal for radio isotope work. The baffle is also made of stainless steel and is easily removable.
Circle No. 46 on Reader Enquiry Coupon

High stability optical benches. Oriel. Many precision optical components mount on to the precision high stability optical benches. These utilise a unique dovetail rail with spring-loaded, wobble-free carriers. Low 'stiction' surfaces allow extremely smooth positioning of the carriers. With care these carriers can be positioned to a precision of 0.001 inch (25 μm) without screw adjustments. Most important, when the carrier locking screw is tightened, the carrier does not shift.
Circle No. 47 on Reader Enquiry Coupon

Automatic blood oxygen dissociation analyser. TCS. The continuous oxygen equilibrium curve of blood can be obtained with the TCS ABOD-analyser in 20 min. Less than one drop of whole blood or haemolysate and 20 min recording times are needed to plot a complete oxygen dissociation curve, and thus obtain the P_{50} value of haemoglobin. The curve is obtained by plotting the absorbance difference of haemoglobin as measured at two different wavelengths against the oxygen partial pressure (P_{O_2}) as measured with a Clark type electrode.
Circle No. 48 on Reader Enquiry Coupon

Precision saw. Anshaw. The Model 7 precision saw from Anshaw Instruments features a choice of two types of quickly interchangeable cutting heads; wire or disk. The wire cutting head was designed to overcome the problem of damage introduced into crystal structures by normal cutting methods. It uses a 0.2 mm diameter, diamond impregnated, continuous wire 'blade' which can be run at speeds up to 10 m s^{-1} and will cut fragile samples, as well as crystals, in practically all materials. The disk cutting head uses standard cutting disks of 100 mm diameter, either diamond or silicon carbide for all precision sawing, including metals, where the extreme sensitivity of the wire saw is not necessary. Various accessories are available, including a precision Goniometer for the orientation and cutting of crystals and also an attachment for automatically cutting circles and cylinders from crystals and hard, brittle materials. The saw has fully controllable cutting speeds and a cross-slide calibrated to 0.1 mm.
Circle No. 49 on Reader Enquiry Coupon



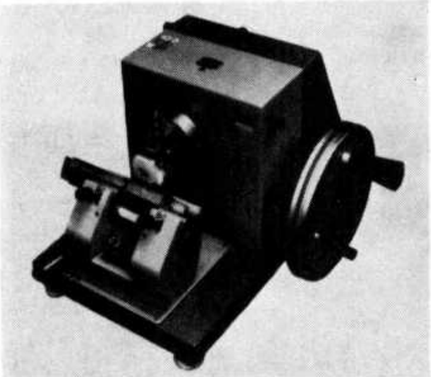
Schoeffel SF 770 and FS 970.

Continuously variable wavelength absorption Monitor/Fluorescence Detector. Schoeffel. By connecting the two units, the SF 770 and FS 970, in series and carefully monitoring the results, confirmation of the spectral characteristics of a compound can be accurately achieved. Determination of the most sensitive method of measurement (absorption or fluorescence) can be made. Extraction of valuable information regarding compounds not clearly separated from each other can be achieved when comparing separate recordings. Both units employ a high energy, low stray light monochromator and offer a selection of light sources (deuterium and tungsten). Both have solid state, miniaturised, modular design.
Circle No. 50 on Reader Enquiry Coupon

Scale culture apparatus. New Brunswick. The MultiGen, an alternative to the spinner flask procedure, is a 1 l or 2 l culture apparatus that can be converted for use with either microbial or suspension cultures. Fully integrated for the control of temperature, agitation, aeration, *p*H and dissolved O_2, the MultiGen also provides facilities for the introduction of sparged air or a gas overlay and includes facilities for heating and cooling, turbine agitation or marine blade stirring. A magnetically coupled drive provides very gentle stirring to vigorous agitation and aeration while an electronic controller ensures reproducible speeds in a range up to 1,000 r.p.m. Precise temperature control from below ambient to 60 °C is achieved by a solid-state thermistor regulator; control accuracy is better than ±0.2 °C. The MultiGen is equipped with its own air supply for aerobic studies. For anaerobic investigations, the pump can be switched off so that CO_2 or other gases may be introduced into the vessel.
Circle No. 51 on Reader Enquiry Coupon

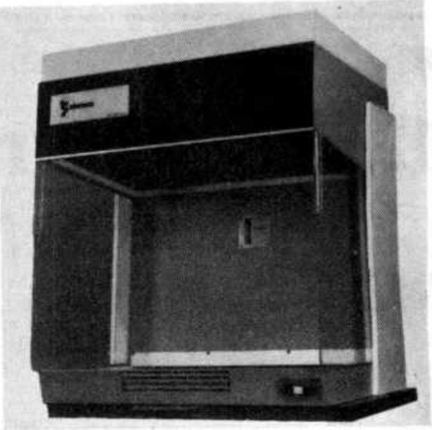
Laboratory sample coater. R. B. Electronics Ltd. A new precious metal coater designed for use in laboratories, the EMS 76 is a compact bench top instrument which diffuses a transparent layer of gold on to non-metallic laboratory samples to enable them to be scanned by an electron microscope. The unit has solid state electronics and incorporates an efficient vacuum gauge which overcomes drift and contamination problems. The 'sputtering' method of coating eliminates the need for rotation or orbital motions. A typical coating time is 2 min for 300–400 Å of gold and the total cycle time is less than 5 min. Virtually any material can be coated, but it is ideal for use on samples of plant or animal tissue, fungus, synthetic fibres and plastics.
Circle No. 52 on Reader Enquiry Coupon

Programmable electronic balance. Sartorius. Model 3802 MP has a weighing range of 6,000 g with 0.1 g readability, operates on the principle of electromagnetic force compensation, and incorporates a microprocessor with a fixed programme to cater for specific problems of weighing. The pan (240× 295 mm) is loaded and the weight read off in less than 2 s. The electronic memory of the balance stores each tare weight automatically after the tare sensor has been touched, while any other required random weights (e.g. standards for check-weighing or batch-weighing) can be similarly stored; only deviations from the standard will then be indicated. The 3802 MP can be used with data processors and control units such as printers, tape punchers, calculators, batch counters, recorders and remote readouts.
Circle No. 53 on Reader Enquiry Coupon



Reichert-Jung disposable knife holder.

Disposable knife holder. Reichert-Jung UK. A new disposable knife holder which fits AO, Reichert, Jung and virtually all other microtomes, overcomes the two most usual objections to the use of disposable blades—the questions of rigidity and adjustment of cutting angle. The holder is of the same size and general cross sectional profile as a standard non-disposable knife, allowing the normal angle adjusters on the microtome to be used. The disposable blade seating and gripping mechanism are so designed that the complete blade/holder assembly is extremely rigid enabling hard specimens to be sectioned without judder.
Circle No. 54 on Reader Enquiry Coupon



Pathfinder downflow work station.

Downflow work station. Pathfinder. The new R133/6V range of bench mounted lamina downflow work stations are for use in medical research, diagnostic analysis, haematology and pathological laboratories, and the pharmaceutical, electronics and space industries. The R133/6V features easy operator access and top mounted, high efficiency filters, has air flow or 90 ft min^{-1} (0.45 m s^{-1}) and is fully recirculating with 10% front air make-up. The fully balanced, maintenance free fan system has been specially designed to ensure minimum vibration and a low sound level in the region of 60 dB (A scale) at 1 m. The largest unit in the range measures 1200 mm high by 1924 mm wide by 903 mm deep. The work surface is perforated stainless steel and side screens are of clear Perspex.
Circle No. 55 on Reader Enquiry Coupon

Conductivity meter. Philips. The PW 9505 has a choice of 10 standard measuring ranges, from 0–3 μS cm^{-1} up to 0–100 mS cm^{-1} for which the new Philips plastic conductivity cell PW 9514/60 should be used. By using a specially developed screened cable supplied as standard with the instrument, together with certain Philips glass conductivity cells, the operator can cover the eight middle ranges. A large, easily read scale minimises reading errors, and gives high accuracy with 0.5% class meter. Facilities are provided for conductimetric titrations, reference measurements and ordinary resistance measurements. Reference temperature is normally 20 °C, but it can be extended to 25 °C with a minor modification.

The instrument provides a choice of manual or automatic temperature compensation. The manual mode covers the range from 0 to 50 °C. The automatic mode, carried out with Pt100/1 resistance thermometer or reference cell, covers the range from 0 to 120 °C.
Circle No. 56 on Reader Enquiry Coupon

nature

11 August 1977

A suitable case for education

We are back in the what's-wrong-with-British-industry business again. After a lull of every bit of twelve months during which there has not been a single weighty document for us to consider in all its worthy detail, the Department of Industry has, with some connivance from the Department of Education and Science, put out a discussion paper, *Industry, education and management* (free from the Department of Industry), which is aimed to "prompt discussion and, more important, action" on the quality of industrial management. The paper fully acknowledges that the poor productivity showing of British industry is by no means exclusively the result of bad management, but tries at least to isolate just a limited number of aspects of the problem—those concerned with technological management by graduates in Britain's manufacturing industries.

If the paper is familiar stuff in many ways, being (so it seems) a distillation of generally expressed views and well-known pieces of information rather than an expression of radically new thoughts, it still serves a useful purpose of confronting the nation yet again with the woeful way in which it doesn't care about engineers and engineering, somehow regarding the creation of wealth as bing a relatively inferior occupation, to be avoided if anything else (preferably involving only paperwork) can be found. The statistics tell the story starkly. As recently as 1969, 40% of graduates entering home employment (excluding the medical sciences) went into manufacturing industries. Now the figure hovers around 25%. At the same time growth in the number of first-degree graduates in science and engineering all but stopped after 1968. Furthermore, tables of after-tax salaries adjusted, as well as can be, for the cost of living in different countries show that top executives on the manufacturing and engineering side are consistently worse paid in the UK than in any other European country. Managerial salaries in industry have lagged very seriously behind inflation and as a result of that and very heavy taxation the salary differential between top executives and average weekly-wage earners is much lower in the UK than anywhere else in Europe save super-egalitarian Sweden.

Nor is there much but gloom in those perceptions of industry which cannot be turned into figures. Television nightly presents the British industrial scene as a battleground between aloof management and irresponsible unions over issues of the most trifling character. Companies are often thought to be run by boards remote from the factory both gographically and in comprehension. Industrial jobs are reckoned to be much more vulnerable than those in the professions, government or academe. Who then is for manufacturing industry; most of all who wants to be on the production side—the Cinderella of the engineering profession?

All of this has, in one form or another, been talked about *ad nauseam* in the past few years without as yet any perceptible improvements, and more doses of it are on the way with a British Association report and then the government's own enquiry into the status of engineers. But in the end it is the educational system which must take up the running, if only because it has a flexibility within it that industry does not have. The first step must be to persuade more intelligent young people at least to sample engineering at school and in university. There seems to be some evidence that young people go into engineering on the basis of the impression that local industry makes on them, either through family connections or simply through exposure to nearby activity. It ought then to be the duty of schools to make sure that local industry really makes itself accessible and explains what it is about to schoolchildren.

Schools cannot expect to do this without one or two of their staff having a more than passing acquaintance with industry themselves. And universities should make sure that engineering is better taught, with richer experience. How many students' summers are wasted by selling ice-cream when they should be learning what industry is about by being in it—and how many universities go to much trouble to persuade industry to find summer jobs for their students? ☐

The world balance: judge with care

Robert Press delivers his verdict on the 1977 Yearbook of the Stockholm International Peace Research Institute.

THE 1977 Yearbook has not avoided the dangers about which SIPRI was warned in *Nature* editorials in 1975 (17 April) and 1976 (8 July). It must be regretted by any objective student in the armament and disarmament community that such an otherwise impressive compilation of military statistics should at times depart from the detachment and neutrality that SIPRI could so usefully provide. Various points are made and opinions or beliefs are expressed which, in the absence of being argued through, have too often an appearance of slanting objective information in a manner which one had hoped an organisation with SIPRI's purpose would avoid.

In analysing the non-nuclear issues, the 1977 Yearbook appears to be very much more concerned with the armaments side of the coin than with the disarmament side. The dimensions of a problem are often stated and supported by a wealth of statistical information but with little or nothing about what is being done to tackle the problem. The Yearbook gives only cursory coverage to the work of the Conference of the Committee on Disarmament (CCD); some analysis of the 1976 sessions would have been useful to the reader rather than merely five brief references to CCD plus lists of working papers tabled in 1976. A SIPRI assessment of the UN Special Session on Disarmament, due to take place in New York in May–June 1978 would also have been welcome. Although this is of great importance as a non-aligned initiative, it is disposed of in a three line reference in an appendix and it is likely to be over by the time the 1978 Yearbook appears.

The Yearbook performs a most useful service in publishing arms trade registers. The section on world military expenditure contains a mass of detailed and interesting data, though lacking in steps to reduce it. One looks in vain, in the index, for a reference to the UN Reduction of Military Budgets (ROMB) exercise, or for the Economic and Social Consequences of the Arms Race (ESCAR). Neither is there reference to a significant US piece of legislation in the International Security Assistance and Arms Export Control Act of June 1976, nor to the UN Secretary-General's study in an 1976 experts' report on the measurement and international reporting of military expenditures. Also lacking is a reference to the Japanese initiative (in the 1976 United Nations General Assembly) with a draft resolution calling for a UN study of international arms transfers. It is significant that this resolution was supported by the West but frustrated by the non-aligned countries, many of whom resent what they considered to be attempts to restrict their capacity to defend themselves.

The most significant point which emerges from a study of the tables on pages 222–3 is the increase in the military expenditure of Third World countries, whose share of global military expenditure appears to have increased from 6% in 1966 to 15% in 1976. One would have welcomed some explanation of this rapid growth. It is not all due to increased arms transfers since these still represent only 14.5% of the Third World's military expenditure. Granted that the arms trade with Third World countries is the area where the greatest increase has occurred, inclusion of trade between industrialised nations would not only have been useful to complete the global picture but would have provided a useful basis for comparison between SIPRI's figures and the United States Arms Control and Disarmament Agency (ACDA) annual volume on world military expenditures and arms transfers.

In discussing developments in nuclear arsenals it is asserted that "the most dangerous development in strategic weapons is the continuous improvement of the accuracy of warhead delivery". One would have welcomed an argued defence for this assertion against the case for greatest accuracy possible for all targets other than cities. In this connection it is arguable (but again not argued) whether, as asserted, military R&D is the "one activity which makes possible the arms race" or whether such R&D is an inevitable consequence of a prior political situation. The latter possibility appears to be recognised in the chapter on international nuclear transactions where it is said that a shift of emphasis "towards the root causes of nuclear proliferation is urgently needed" and that "the best way to slow down nuclear proliferation would be for the nuclear weapon states to show by their actions that they are willing to downgrade the political and military role of nuclear weapons".

The chapter dealing with the 'main events and concerns of the year' (1976) inevitably refers to "the new surge of concern about the possible proliferation of nuclear weapons to countries which do not already have these weapons" and it looks upon the year as a "a particularly meagre year for efforts to slow down the Soviet–US arms race to limit armaments". Even the Peaceful Nuclear Explosions Treaty, signed in 1976, would in SIPRI's view, like the 1974 Threshold Test Ban Treaty, be better to remain unratified since it may only delay a comprehensive test ban. One can readily agree that any treaty which allows PNE to continue cannot be truly described as a comprehensive test ban treaty.

Given the importance of the whole issue of nuclear weapon proliferation, the confusion and emotion that arises in discussing the inter-relationship of civil nuclear capability and the potential diversion to military application, the Yearbook's treatment of the proliferation issue is at times less precise and objective than one could hope for from SIPRI. For example, in a reference to the proliferation of uranium enrichment technology to non-nuclear weapon countries,

Keystone

Launching the Cruise Missile

“in particular to FR Germany, the Netherlands and South Africa”, the Yearbook fails to point out that these three countries were themselves simultaneously developing isotope separation technology. This may be “proliferation” in one sense but not in the generally accepted sense of commercial transfer of know-how and equipment from nuclear haves to have-nots.

It is claimed that while the Soviet Union followed the USA, UK and Canada in negotiating bilateral agreements, they did so in “a more cautious manner”, following their dispute with China, and they have been “rather restricted in their nuclear transactions . . . whereas some Western European countries have adopted more aggressive commercial practices”. Yet in the same chapter it is noted that when Canada permanently cut off its nuclear sales to India, two years after the Indian nuclear explosion (1974), “the Soviet Union agreed to sell 200 tons of heavy water to India instead”. There is no mention that this action was taken before a safeguards agreement with IAEA had been negotiated. The Yearbook has no hesitation in pointing to a USA offer “to sell nuclear reactors to Israel and Egypt even though these States are not subject to NPT Safeguards” without adding, for the readers’ information, anything about the offer being subject to IAEA or US safeguards. Reference to the major nuclear suppliers’ efforts to minimise the risk of diversion of nuclear equipment and material is accompanied by the comment “which they are so eager to supply”, without any acknowledgement of those countries’ obligations under the NPT.

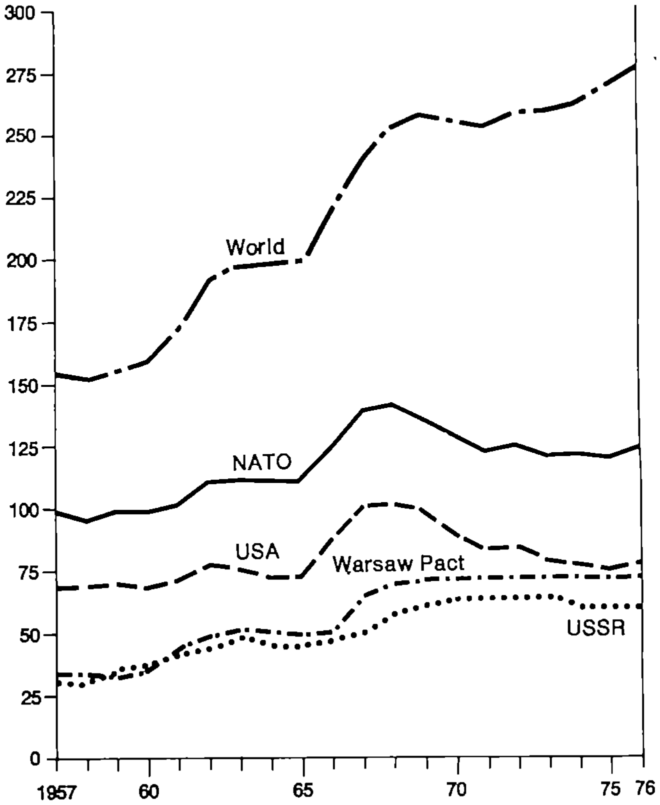
Incidentally, under a sub-heading of nuclear explosives as by-products of a peaceful nuclear power programme, in Fig. 1.1, one wonders about the value of the statement: “The marginal cost of so producing a nuclear explosive device may be no more than a few hundred thousand dollars”. The reader may well wonder whether this is an informed estimate and what is meant by “marginal cost”; if not an informed estimate, does the statement render any positive service to the cause of non-proliferation? However, in this context, a more realistic note is struck when it is observed that “a lack of access to a commercial reprocessing plant would not necessarily prevent the proliferation of nuclear weapons to countries which take the decision to acquire them directly”.

On ‘the plutonium problem’, the Yearbook falls into the usual trap of failing to separate the ‘nth country’ problem from the potential ‘terrorist’ problem and therefore fails to distinguish between the different kinds of risks arising and then of course in the different kinds of safeguards to be adopted. In a reference to breeder reactors it cites the usual “dangers” arising from their preferred fuel being “plutonium of weapons grade” while appearing to underplay the contribution that any grade of plutonium can make for the same purposes or the relative attractiveness of enriched uranium for bomb making. In general, one would have preferred SIPRI to examine various aspects of the plutonium and breeder issues in a perspective which would inform and admit a reader’s own judgment to be made rather than appearing to lean towards an anti-breeder if not an anti-nuclear viewpoint. For example, does SIPRI have a view on whether proliferation stemming from breeders can be forestalled by proper design, both technical and institutional; if it can, should proliferation-resistant breeder systems be set aside in face of forecast world energy requirements and particularly in face of the forecast ‘CO_2 problem’ arising from the burning of fossil fuels?

From the point of view of avoiding unnecessary alarm, or providing the neutral reader with a clear perspective, the section on ‘Accidents of nuclear weapon systems’ is one of the most unsatisfactory in the Yearbook. Whether the picture is presented as “substantially more serious accidents involving US nuclear weapons than the public were aware of” or “about one every two and a half months”, the text on these is too often laced with such expressions as “it is reasonable to assume . . .”; “it is not unlikely”; “there is thus even some possibility . . .”. These are the phrases which may leave some readers with an impression of frequent nuclear accidents when only a related weapons ‘system’ or vehicle may have been involved in either a major or minor accident, or even only an incident of some kind, and where “there is no record of any of them having involved nuclear weapons in transport” or where “there is no indication of whether or not any of these accidents involved nuclear weapons”.

World military expenditure, 1957-76

US $ thousand million, (1973 prices and 1973 exchange rates)



Source: SIPRI data

The text specifically states that “there is still no direct evidence of involvement of nuclear weapons in the case of the French and British weapons systems”, yet the same chapter contains a table under the heading of “British nuclear weapon incidents”. Admittedly this particular inclusion is explained away in a series of footnotes but, even within SIPRI’s own justification for such presentation, would it not have been more helpful and reassuring to the reader to make the point that in the West there is no chance of a nuclear accident, involving even the slightest escape of radioactivity, being concealed, and that on this basis the record is one pointing to the very high standards of safety employed? In any event, in the light of the evidence presented, it is difficult to accept what the conclusions offer as being “clear” or “incontestable fact”.

These various observations in respect of SIPRI’s presentation of both non-nuclear and nuclear issues are made to illustrate the need for careful examination of various assumptions made in SIPRI’s analysis before accepting the general conclusions drawn. At the same time they illustrate once again the dangers about which *Nature* editorials have warned in previous years and, hopefully, will encourage SIPRI to avoid a continuing trend that could so easily destroy the value of a reliable and objective reference work for which the need is great. □

Man-made process

Chris Sherwell reports on a study of man's involvement in the spreading desert

HOWEVER discomforting it is for Western man to learn that he wastefully squanders his resources, his lesson is not nearly as painful as the one being learned by his contemporary living in the arid zones of the underdeveloped world. For there, that most precious and finite of resources, the land itself, is actually being lost, and at an alarming rate.

Some wealthy countries experience the same problem. Fifty million of the 163 million acres managed by the United States Bureau of Land Management, for example, are in 'poor' or 'bad' condition. But the US has the wherewithal to combat the phenomenon: the causes of desertification are understood imperfectly, but well enough for something to be done.

The real problem is conjuring out of nothing the political will to implement a comprehensive plan that deals with the poor countries' marginal lands. It is a problem which will confront the massive UN Conference on Desertification starting at the end of this month in Nairobi, when just such a plan will be drawn up. As with past jamborees of this sort—on Water earlier this year, Human Settlements last year or the Environment in 1972—a flood of papers is besieging the conference. One of the more important was published last weekend by the Washington-based Worldwatch Institute*.

Entitled *Spreading Deserts—The Hand of Man*, it is, as its title suggests, unequivocal about causes and solutions. It calls desertification an ecological phenomenon but says it is a human problem. "People cause it, people suffer its consequences, and only people can reverse it." Governments may find it difficult to devote substantial resources to combating a "seemingly long term and nearly invisible problem like ecological deterioration", but the consequences of desertification—undernutrition and famine, unemployment and migration, deepening poverty and human desperation—are "neither distant nor invisible".

Some of the statistics cited in the study confirm the need for action. About 14% of the world's population —630 million people—live on arid or semi-arid lands. Of this number, some 12% live on lands already almost useless and face the loss of their livelihood.

The decline in the quality of the land has meant a decline in the quality of people's diet, chronic ill-health, swollen ranks of urban migrants and lost economic potential. Indeed, cumulative degradation of rangelands and nonirrigated farmlands in the arid zones has, according to the study, held their combined annual productivity "more than $12 billion below its potential level"; of sixteen underdeveloped countries in the arid areas, for example, only two significantly increased their per capita grain output between 1950 and 1975.

This decline is more popularly portrayed in another way. Along the southern fringe of the Sahara alone, 650,000 km^2 of land once suitable for agriculture and grazing has been lost in the past 50 years. Some 100,000 ha of range and cropland are lost to desert each year along the Sahara's northern margins. Elsewhere—in the Middle East and in Western Asia—the process's hold is "particularly long-lived and advanced". It is also taking place, as the accompanying map shows, in many of the western hemisphere's drier regions.

However, although natural desert encroachment is a real enough threat in some areas, the process of desertification is more correctly one where human action causes the desert to spread. A product of land-use patterns as well as climatic fluctuations, desertification will occur where land is abused in arid and semi-arid zones regardless of the actual proximity of the climatically-created deserts. The effects of severe and prolonged land abuse are, of course, intensified by extended droughts. But to review the current theories and knowledge on world climate, says the study, is not only to "sense the urgent need for research efforts" on climatic trends; it is also to "see without question that people and their livestock are helping to downgrade the carrying capacity of arid lands and to create new spots of desert".

Desertification is not new; what makes today's problem especially acute, says the study, is the scale of suffering when the rains fail, and the scale of destructive human pressures. Rudimentary modern medicine has helped produce the 3%-a-year increase in population prevailing in the areas, which means more people are reliant upon the pastures and croplands there. At the same time the modern political and social order has grown inimical to the existence of the desert dweller, restricting nomadic groups who are well-adjusted to a harsh environment and encouraging sedentary agriculture.

The results are all too obvious. More people with more herds cultivate more areas to feed more mouths. Soils deteriorate, yields decline, pastures disappear, grazing land grows more distant and land around more intensively used water holes perishes. People become a threat to themselves: what they do to ensure individual survival in the short term is detrimental to the society's long term survival. As the Worldwatch study sees it, social systems and ecosystems are on a collision course; populations in the arid areas have reached the ecological danger point.

The study is also clear about what needs to be done. For a start, herd

Camera Press

Where to from here?

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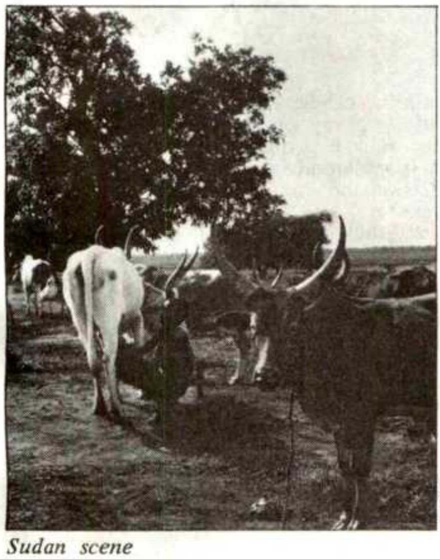
*Eric Eckholm and Lester R. Brown, *Spreading Deserts—The Hand of Man* (Worldwatch Paper 13, Worldwatch Institute, Washington; August 1977).

sizes should be reduced. Livestock should be valued for its quality rather than its abundance. If animal numbers in the Sahel were maintained at half the 1971 level, with modern management techniques the region's output of meat and milk could be double that level. Farming in the sedentary zones should also be improved, to halt its spread onto pasturelands. Farmers need knowledge and equipment to grow sufficient food using the best lands without loss of fertility; back-up for subsistence farmers has hitherto been neglected in favour of cash crops, which have themselves exacerbated the lot of the rural poor.

In addition, couples must perceive the advantages of small families. Population growth, says the study, prohibits a return to the ecologically sustainable rotation systems, so new cropping systems, along with tree-planting programmes, are the only alternative. Also, to urge the resettling of nomads and the killing of goats is to oversimplify: a modernised version of the nomadic way of life may be necessary, with clan leaders, aided by specialised advice, regulating grazing and migration through large herding cooperatives.

A major goal of arid lands development, says the study, must be to prepare peoples and economies to live through the inevitable droughts without trauma. Protection against famine, it says, is not a simple technological problem. Its success depends on nothing less than "the broad shape of national development and the character of the prevailing socio-economic institutions".

Plainly the challenge is great. It is a challenge facing the UN Conference. If that fails, and past experience does not augur well, the people most affected will be left to face it alone. They have done so before. But their chances of successfully continuing to do so are not improving. ☐



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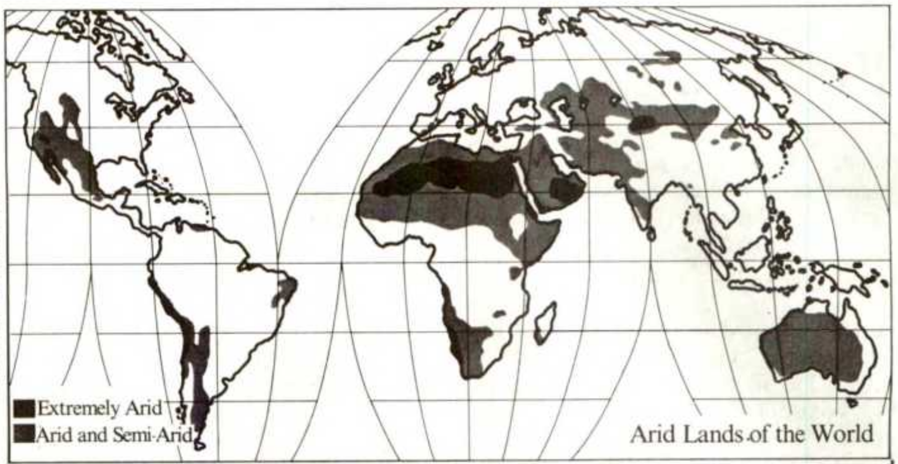
Sudan scene



UNESCO map by P. Meigs

ICARDA gets down to business

THE International Centre for Agricultural Research in the Dry Areas (ICARDA), having shaken off its troubled origins, is poised to embark on an ambitious research programme under the guiding hand of Dr Harry S. Darling, now president of Wye College, who is preparing to move to Beirut as ICARDA's director general.

The most recent of the international agricultural research centres, ICARDA will focus on improving food production in areas with an annual precipitation of 300–800mm that is concentrated largely in the winter months. Its findings will affect a region extending from Morocco to Pakistan with crops covering some 70 million hectares in 20 countries.

ICARDA staff members like to say that the centre "was born running", because it absorbed some of the facilities and personnel of the Arid Lands Agricultural Development (ALAD) programme of the Ford Foundation. But it was soon faced with obstacles that brought it almost to a complete halt. After the decision to establish head offices in Beirut in Spring 1974, war broke out, and most of the staff scattered to Cairo, Aleppo and Damascus. Negotiations with Syria and Iran to obtain land for research stations were conducted chiefly by Canada's International Development Research Centre (IDRC), which had been appointed the executing agency for ICARDA; formal agreements were signed with Syria in June and with Iran in July last year. IDRC also reached an agreement with the Ford Foundation to insure that ALAD activities relevant to ICARDA's mandate, notably the collection, testing and exchange of plant genetic materials, were continued.

On top of its research on the region's crops, ICARDA has been designated as the world international centre for barley, lentils, and broad beans. Barley, one of the world's oldest crops, is particularly important in the region. But wheat is usually preferred because it has a higher gluten content and makes a more cohesive loaf of bread. Barley's two major advantages over wheat, however, should encourage its utilisation as human as well as animal food. One of these is that in regions with less than 500mm of annual rainfall it can outperform wheat; where rainfall is 375 mm or less, it is practically the only cultivable cereal. The other advantage, hinted at by current research, is that barley is susceptible to improvement, notably by an increase in content of lysine, an essential amino acid that is absent or exists only in minimal amounts in most food grains.

At present, barley yields in the Middle East are very low, and four ALAD-ICARDA barley varieties introduced in nurseries in 1976 indicate that performances can be considerably improved. Research is under way to deal with problems of disease susceptibility, lack of winter hardiness, intolerance to high temperatures, and insects. These trials will be undertaken in the ICARDA stations near Aleppo and Tabriz.

The food-legume crops improvement programme also continued in 1976. It consisted mainly in breeding and distributing germ plasma of three major pulses: lentils, broad beans and chickpeas. The major emphasis is on the development of high-yield, stable varieties, and of lines suited to mechanised harvesting. There is a forage-crop improvement programme, and some of the ALAD research work in wheat, maize and grain sorghum is being continued; so is the evaluation of several hundred lines of triticale, a man-made hybrid of wheat and rye.

Next year, ICARDA is expected to expand its activities. These will include the study of environmental systems throughout the region, the establishment of principles on which to base the development of appropriate farming strategies, and research into socio-economic constraints that limit the actual and potential production of existing farming systems. Finally, a training programme to help apply research findings is being set up.

From its inception until the end of last year, ICARDA's total budget barely exceeded $3 million. But now that 'running speed' is being resumed and total staffing has exceeded 50, more funds are required for operations and capital development. Interest in the programme is illustrated by the commitment for 1977 of a total of $4 million, the major contributors being the USA with $1 million, the IDRC and Canada with $600,000, Iran and Saudi Arabia with $500,000 each, and the World Bank and Australia with $220,000 each. Estimated expenditures of $4.85 million for 1978 and $7.77 million for 1979 have been submitted to the Board of Trustees, which is made up of representatives of some of the region's governments, agricultural research centres and donor agencies.

Alexander Dorozynski

Pieces of action

Sandy Grimwade reports from Washington on some of the non-legislative developments concerning recombinant DNA research

ALTHOUGH much of the attention of the followers of the recombinant DNA debate has focused on the progress of the controlling legislation at present under consideration in Congress, activity in research labs and on campuses has not been completely stilled. A major supplier of laboratory chemicals has taken some unprecedented steps to control the use of its restriction enzymes—the enzymes which are used in the preparation of recombinant DNA molecules. A Maryland lawyer has sued the National Institutes of Health (NIH) on behalf of his son to postpone recombinant DNA experiments which he claims are at present illegal. And another group of scientists has petitioned Congress over the proposed legislation.

The firm which has taken a step practically guaranteed to lose it customers while bringing praise from those who favour a cautious approach to recombinant DNA research is Miles Laboratories Inc. Miles has been an important supplier of restriction enzymes for several years, and last year sponsored a symposium on recombinant DNA research. In themselves restriction enzymes can cause no hazard; a competent molecular biologist, moreover, would be able to prepare a private supply with a week or so of work. Miles now requires its customers to appoint a control officer who will be responsible for ensuring that its reagents are used in accordance with the NIH guidelines, and who must sign all orders for such reagents.

A spokesman for Miles said that the firm had taken this step on both moral and legal grounds, and that the restriction will be kept in force until the federal legislation now pending becomes law. Miles is applying this condition to all sales of restriction enzymes on the advice of its lawyers so that the entire burden of responsibility for their use is shifted to the customers. The spokesman admitted that the reaction from scientists to this additional bureaucratic requirement had not been entirely favourable. Its action is unilateral; other companies in the business are apparently not planning to follow suit.

The action of the attorney in Frederick, Maryland, who filed suit under the National Environmental Protection Act on behalf of his 2-year-old son against NIH, was aimed at halting experiments planned to take place at Fort Detrick before publication of an Environmental Impact Statement (EIS). The experiments, which would involve the splicing of segments of the mouse polyoma virus genome as well as the whole genome into plasmids and cloning in *E. coli*, would under the NIH guidelines require the highest level of physical containment, the P4 level. The objective of the experiments is to discover whether the cloned gene segments of the polyoma virus, which causes cancer in mice but has no effect in humans, are expressed in *E. coli* and whether the gene products can raise antibodies in mice treated with the *E. coli* clones.

NIH have agreed to produce an EIS in 30 days, although it is not clear at this point whether the statement will address the potential impact of this particular experiment or recombinant DNA experimentation as a whole. The attorney, Ferdinand Mack, sees the NIH action as a concession that they were ignoring the law requiring the publication of an EIS. In fact the EIS from NIH on the complete recombinant DNA guidelines has been hanging fire for over a year now, a point which does not follow the letter of the National Environment Protection Act. Friends of the Earth have filed a separate injunction enjoining a halt to all federally funded research in this area until the EIS is published.

The latest petition from scientists follows the statement about a month ago from the Gordon Conference on Nucleic Acids urging Congress to drop or tone down the proposed legislation. Signed by 140 scientists attending the Conference on Biological Regulatory Mechanisms—85% of those attending—the new statement was sent to members of Congress as part of a late-starting but vigorous effort by the proponents of recombinant DNA research to urge more lenient legislation. ☐

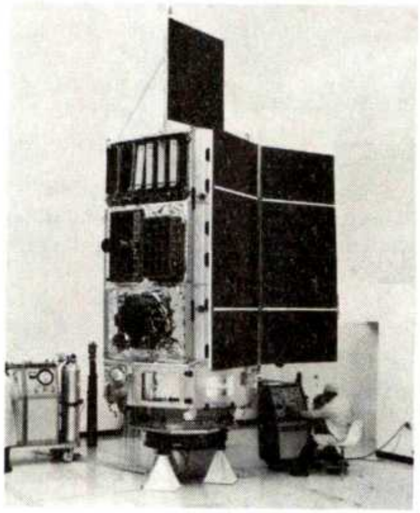
Scanning the universe

X-RAY astronomers are not having much luck at the moment. ESA's decision to schedule Exosat on the Ariane launcher upset the European space community, less because of Ariane's unknown reliability than because of the spectre of budget cuts for other projects and an almost certain delayed start to the mission. Though US astronomers have fared no better in recent months, some improvement may now come with the first satellite of the HEAO mission, which NASA expects to launch this week.

The High Energy Astronomy Observatory (HEAO) project was initiated in 1968. Budget cuts have since halved its size, but still the three-satellite mission involves the heaviest Earth-orbiting satellites ever launched. NASA had hoped to launch the first satellite, HEAO-A, on 15 April, but problems with the spacecraft's gyroscopes caused cancellation at the last moment; re-design and qualification of the gyros have now been completed. Though a launch scheduled for 30 June was missed, lift-off is fixed for the early morning of 12 August.

The HEAO observatories are designed as sensitive high-resolution detectors of X-rays and γ-rays. Building on the success and experience of such missions as Uhuru, Copernicus, Ariel V and SAS–3, HEAO could help resolve controversies over the existence of black holes and the nature of quasars, pulsars and X-ray bursters. No one commits $237 million solely to fashion, however, and the HEAO mission has a solid, professional set of scientific objectives to pursue.

HEAO-A is an all-sky X-ray survey mission. A circular, 445-km high orbit will place it above the absorbing atmosphere and below the damaging radiation belts. During its six-month lifetime it will scan the complete sky and systematically map both discrete X-ray sources and diffuse X-ray emission and absorption. The four experiments on-board provide high resolution (up to 5 arc-seconds) for position and size determinations and good time resolution for investigating the complex behaviour of variable sources. Crude spectral data will also be obtained.



TRW

HEAO-B is due for launch in 1978. Containing a wide-aperture imaging X-ray telescope with a sensitivity 10,000 times greater than any previously flown, it will latch on to specific sources of interest pinned down by HEAO-A and study them in great detail. The mission lifetime is estimated as one year. HEAO-C, another all-sky survey satellite and due for launch in 1979, will systematically scan for cosmic and γ-ray sources.

Stuart Sharrock

USSR

Well provisioned

The Soviet Union is at present going through the rituals of adopting a new constitution. Vera Rich reports

THE DRAFT of the constitution which has been presented to the public and is now the subject of 'discussion' (in effect, eulogy and explanation) in the media is, from a political point of view (State structure, the rights and duties of citizens and so on), little different from the existing version. But there is one interesting feature: the emphasis placed on the State's involvement in Science.

The existing constitution, promulgated in 1936, makes virtually no mention of science. In contrast, the new draft stresses that "in accordance with the requirements of society, the State guarantees the planned development of science and the training of scientific personnel", and the implementation of the results of scientific research in the economy (Art. 26).

A new clause (Art. 48) guarantees "freedom of scientific, technical, and artistic creativity", which is "ensured by the wide development of scientific research, inventive and rationalising activity and the development of art". The same clause pledges state support to "voluntary societies and creative unions", and guarantees the rights of authors and inventors. The "right to work" is now extended (Art. 40) to include the right to choose a profession "in accordance with vocation, capabilities, professional training, education, and in accordance with State needs".

A somewhat ambiguous reference to science in the 1936 version has now been clarified: instead of guaranteeing the right of asylum to foreign citizens persecuted for "defending the interests of the working people, or for scientific activities", which suggested that the Soviet Union hoped to play host to latterday Galileos, the reference is now to "progressive social, political, scientific, or some other creative activity".

The new references to science clearly indicate a serious government commitment. To stress their importance, *Pravda* recently published a long statement by scientists of the Siberian Branch of the Soviet Academy of Sciences, a body which, being less than 20 years old, can well serve to express the viewpoint of the modern scientific community. In effect, the statement proved to be little more than a eulogy of the Soviet system of "guaranteed employment".

Not surprisingly, the eulogies are not echoed by dissident and refusnik opinion. Dr Veniamin Fain, a physicist who was allowed to emigrate in June, recently told *Nature* that he was very doubtful that the new clauses would bring about any major changes as regards academic freedom, and the status of refusnik scientists.

The picture he describes, of scientists dismissed from their posts and forced to find work as janitors or lift-attendants simply because they wish to leave the country, accords ill with the new pledge of choice of profession in accordance with one's education. And the history of the Voronel-Azbel seminar hardly reflects the "support" pledged to "voluntary [scientific] societies". Nevertheless, a right once granted on paper can form an important rallying point for those who wish to press for its implementation. □

EEC

Halocarbon decision

The European Commission has taken a first cautious and hesitant step towards restricting the use of halocarbons in EEC countries. A special correspondent reports

THOUGH not convinced that halocarbons are harmful, the European Commission is sufficiently impressed with the evidence so far, particularly from the USA, that it has decided to ask environment ministers in the Nine to ban any increase in manufacturing capacity for the halocarbons F-11 and F-12. The Commission also wants immediate measures to "encourage" industry to avoid any leaks of these gases, and to "encourage" industry to intensify research into possible substitutes and alternative methods. And it believes research into the effect of the gas on the environment should be stepped up, with more cooperation and coordination between researchers in the member states.

The Commission has been holding meetings with national experts since January. If all goes according to plan, the EEC's Economic and Social Committee and the European Parliament will give their views on the Commission's proposals in September and October, and the environment ministers will make their decision in November. The Commission will then in the second half of next year assess the effects of halocarbons on man and the environment and draw up an EEC policy on the subject. Such a policy would need the approval of the ministers of the member countries.

In the meantime the Commission will see if it should try to co-ordinate the work, or if perhaps it should give limited aid under its programme of indirect action. European research at present is concentrated on measuring, on Earth and *in situ*, concentrations of the minor constituents of the atmosphere, studies of spectral properties and reaction speeds and development of mathematical models. □

ARGENTINA

Scientist's plight

ELENA H. SEVILLA is the latest Argentinian scientist whose plight has come to public attention. Senora Sevilla is a 29-year-old atomic physicist who had been teaching at the University of the South in Bariloche and doing research at the Balseiro Institute.

She was arrested in November 1975, just five days after having had a Caesarian operation and whilst still in hospital. Her husband had been arrested two months earlier. Lack of evidence against her led to her acquittal in a court in Rawson in January 1976, but since then she has been held in preventive detention "at the disposal of the executive power". She has been moved several times from prison to prison, most recently to the Villa Devoto in Buenos Aires. There she shares a cell with 20 other women in conditions which are by no means good. Diet causes great concern, and prisoners, whilst allowed to receive three books each month, may not have university textbooks or books related to economics, history, chemistry, physics or languages.

In December 1976, Senora Sevilla applied for permission to leave the country under Article 23 of the Constitution, which allows for those held in preventive detention without charge or trial to opt for exile if they so wish. She has never received a reply. According to Amnesty International, Elena Sevilla has never been politically active. Amnesty asks fellow scientists to write on her behalf to the President, General Jorge Rafael Videla, Casa Rosada, Buenos Aires, with a copy to the local Argentinian Embassy.

correspondence

MLS international standard

Sir,—It is with regret that I report an error in the article 'Astronomy's own cloud' (9 June, page 478). The Australian system, 'Interscan', one of several Microwave Landing System (MLS) proposals, has not been adopted as the next international standard, and has not (yet) beaten all comers. The matter is, sadly, complicated if not bedevilled by partisan politics and if you were to read certain American reports, for example *Microwave Systems News* (June 1977, page 33) you would learn: "US Landing System going world wide".

The pedantic, boring truth is that the All Weather Operations Panel of the International Civil Aviation Organisation (ICAO) met in Montreal last March to consider the various MLS proposals. They recommended by a narrow majority vote (6–4), that the 'scanning-beam' (US–Australian) MLS scheme be submitted to the ICAO Air Navigation Commission for consideration for adoption as the international standard at an ICAO meeting to be called later this or early next year. Recommendation for adoption—no more, no less.

It is not altogether clear from the generally accessible published material just what the differences between the American system and the Australian 'Interscan' are. We can certainly say that the Australian idea was taken over by the Americans, whose own originally proposed schemes were demonstrated to be less than competitive. In this sense the Australians do indeed have cause for pride.

One must not forget that the Germans and the English both have advanced MLS proposals, and that the selection at Montreal of the US–Australian scheme was perhaps helped more by procedural niceties than by an honourable and convincing demonstration of the inferiority of the European work (*Aviation Week and Space Technology*, 28 March, page 26 et seq., *Frankfurter Allgemeine Zeitung*, 14 April, page 8). This was the subject of objections at the time and the reason for protest abstentions in various votes during the meeting.

One cannot altogether blame the Australians for blowing their own trumpet since they have suffered sorely in publicity at the expense of the Americans—their trumpet should nevertheless play the written notes and not extemporise.

It is to be expected that the European interests in MLS will pursue vigorously a re-appraisal of the Montreal deliberations, with a view to demonstrating the technical superiority of one of the systems over the others —a demonstration which has not yet been attempted.

Yours faithfully,

David Ll. Hills
Max-Planck-Institut für
Radioastronomie, Bonn

Wind power for the UK

Sir,—Dr Clement states that my estimates of wind-power are "grossly exaggerated" and that the environmental impact would be unacceptable. He compares my results with those of the recent Department of Energy report *The prospects for the generation of electricity from wind-energy in the United Kingdom* which considers the installation of windmills on hill-top sites (many of which would undoubtedly be environmentally unacceptable besides involving greater construction and transmission costs) and within a 15 km coastal belt. The total area involved in deriving their "upper limit on energy" is confined to about 1% of the UK land area. The more dispersed system which I discussed covers—as Dr Clement correctly computes—some 5×10^4 km^2 of land area, reduces the transmission costs per kW, and provides 20 times the energy.

The impact of wind generators on other land uses such as agriculture is minimal, and the visual impact must be judged in relation to that associated with any other scheme capable of providing comparable useful energy. If, for example, it were proposed to provide the UK energy gap predicted by the end of the century by the construction of new nuclear capacity on coastal sites, much of the south and east coasts of the country would require an average density of nuclear stations corresponding to about 0.2 GW km^{-1} of coastline. If, as in my wind example, environmental and other objections permit only about one-third of the coastline to be so used, these areas will require about one 275 kV twin-wire transmission line running inland for every 2 km of coastline. At 4 towers per km the density of pylons (whose construction is very similar to that of the windmills I considered) is then about twice that of the windmills. The nuclear stations themselves would of course involve both visual and other environmental problems as well.

Dr Clement seems to prefer to ignore the energy problems which we, and other countries, now face; perhaps he believes—as the Secretary of State has recently stated—that 13 GW of additional nuclear capacity (half in FBRs) would be more than adequate to meet our energy needs "in the foreseeable future" (*Guardian*, 3 June). Although this capacity would replace the oil-fired generating stations now in use (11 GW), it could make little contribution to the much larger energy demand at present provided by oil and gas for transport, industry and the heating of buildings. Owing to the large fluctuations in the latter, an installed capacity of 200–250 GW would be needed by the end of the century.

A nuclear capacity of 13 GW may indeed represent what it is feasible to build on this time scale, and if so it is clearly essential both to introduce every possible economy measure now and to proceed as fast as possible with the development and installation of alternative sources capable of making a more significant contribution. Wind-power is the most highly developed, has a large potential and is the cheapest, although wave, new forms of tidal and direct solar energy may also be important.

In comparing the cost of wind energy with that derived from nuclear and fossil fuel stations the Department of Energy report has considered only the replacement of base-load electricity, rather than the much more important energy gap discussed above. Owing to the large fluctuations in this energy demand, it is not permissible, as the report has done, to include the interest on capital for wind generators but exclude it for conventional systems, and the high costs they derive for wind-power are not relevant; the actual costs, both capital and running, for producing a given useful energy from wind are about one-third those of a nuclear system.

Yours faithfully,

Martin Ryle
Cavendish Laboratory,
Madingley Road,
Cambridge, UK

news and views

Enzymoimmunoassay: techniques and uses

from J. Landon

IMMUNOASSAYS are being used increasingly in clinical practice to detect and measure antigen concentrations in biological fluids Some procedures do not employ a labelled reactant, including radial immunodiffusion, rocket immunoelectrophoresis and various automated systems. They are, however, applicable only to proteins, and although simple and precise have limited sensitivity and need high concentrations of monospecific antisera. In 1960 Yalow and Berson introduced a radioimmunoassay (RIA), to determine circulating insulin levels, using ^{131}I-labelled insulin and appropriate antiserum. Use of a radioisotope for labelling a reactant overcame the need for monospecific antisera, improved sensitivity markedly and enabled haptens as well as proteins to be assayed.

Many other possible labels have been studied in recent years, including enzymes, fluorescent or chemiluminescent molecules, viruses, proteins, particles and free radicals. Enzyme labelling has been the subject of most work and provides a feasible alternative to radioimmunoassay. Various names are current, including enzymoimmunoassay (EIA), enzyme-linked immunoassay and enzyme-linked immunosorbent assay (ELISA). The term EIA is preferred because of its similarity to the term radioimmunoassay and because it prevents confusion with the measurement of enzymes by radioimmunoassay.

Advantages and disadvantages

Reasons for the emphasis given to enzyme labelling include its previous applications in histochemistry and cytochemistry; the availability and relative cheapness of many enzymes and of manual and automated systems for their assay; the long shelf life of the labelled product (which can be measured in years); lack of hazards during labelling; and a sensitivity, specificity and applicability similar to radioimmunoassay. Nonetheless, the additional step of determining enzyme activity prolongs the assay and increases imprecision, while the amplification made possible by the use of an enzyme to attain sensitivity will also magnify any error. Furthermore, biological samples may themselves contain enzymes with similar biological effects which can cause a high and variable background, and other factors such as haemolysis may affect the result.

The ultimate success of EIA will depend on its merits as compared with the widely used radioimmunoassay. The relative merits of radioisotopes and of enzymes for labelling has recently been surveyed by Ekins (*Lancet* **ii** 176; 1976). Briefly, RIA is relatively inexpensive, very sensitive and precise, and the sample provides no background signal. But isotopically-labelled reactants have some disadvantages including a slight health hazard associated with their production, and a limited shelf life. Short shelf life poses considerable commercial problems since it necessitates frequent radioiodination and increases costs of production, quality control and distribution.

Analytical techniques

Various procedures using enzyme-labelled antigen or antibody to assay antigens have been developed. The reactant is labelled with one of a wide variety of suitable enzymes (acetylcholinesterase, alkaline phosphatase, malate dehydrogenase, peroxidase and β-galactosidase, for example) (see Sharpé *et al. Clin. Chem.* **22**, 733, 1976; Wisdom *Clin. Chem* **22**, 1243; 1976). At the end of the assay the enzyme-labelled reactant is easily measured by reaction with substrate.

Techniques using labelled antigens

One approach involves a separation step and is similar to RIA; enzyme-labelled antigen and the sample are incubated with a limited amount of specific antibody and the proportion of label distributed between antibody-bound and free fractions gives, by comparison with a set of standards, the concentration of antigen in the initial sample. Antibody-bound and free fractions may be separated by adding a second antibody, or by the use of solid-phase systems in which the first antibody is linked to an insoluble matrix. A combination of these two methods, in which the second antibody is covalently coupled to cellulose—the double antibody solid phase system (DASP)—has been successful.

The separation step in an EIA can serve a second important function, namely to separate the exogenous enzyme being used as the label from any endogenous enzymes in the sample with similar biological effects or from any other constituents in the sample, such as enzyme inhibitors, which could influence the signal.

The first EIA was developed for immunoglobulin E by Engvall and her colleagues (*Immunochemistry* **8**, 871; 1971) and assays have now been described for a wide range of antigens including albumin, carcinoembryonic antigen, human placental lactogen, insulin, cortisol, total oestrogens and testosterone.

The separation step can be avoided in some EIA. In one category, which can be termed 'quenching' assays, there is steric hindrance by the antibody in the bound fraction of the reaction between the substrate and active site of the enzyme (Rubenstein *et al. Biochem. biophys. Res. Commun.* **47**, 873; 1976). Thus only the free fraction retains enzyme activity. Use of enzymes such as malate dehydrogenase and glucose-6-phosphate dehydrogenase that require cofactors, obviously enhances the potential for steric hindrance. Assays of this type have been developed for various haptens, including several drugs of abuse, anti-epileptic drugs and digoxin and have been applied to urine, saliva and plasma.

Alternatively, assays exist of the

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'enhancement' type in which antibody binding potentiates enzyme activity. Such an assay has been described for thyroxine using a thyroxine derivative covalently linked to malate dehydrogenase such that the enzyme has little activity. Enzyme activity increases markedly after adding anti-thyroxine antibodies (Ullman *et al. Clin Chem.* **21,** 1011; 1975). This assay has also been incorporated onto a multichannel biochemical profiling instrument (Galen & Forman *Clin. Chem.* **23,** 119; 1977).

The time saved and the precision gained by avoiding separation compensates for the enzyme detection step and such assays are being increasingly used. This approach is, however, probably not applicable directly to the assay of proteins, and problems due to comparable enzymes and other constituents in the sample may limit the sensitivity that can be achieved.

Techniques using labelled antibody
The standard approach is to react the target antigen with excess labelled specific antibody and to remove unreacted labelled antibody by excess antigen covalently linked to a solid phase so that the enzyme activity associated with the antigen in solution is directly related to the antigen concentration (Maiolini *et al. J. Immun. Methods* **6,** 355; 1975; Barbour *J. Immun. Methods* **11,** 15; 1976).

Sandwich technique: this approach was first introduced by Wide in 1971, using radioisotopic labelling, and is applicable to the assay of large molecules with more than one antigenic determinant. It is designed to improve specificity by excluding fragments of the molecule from influencing the results. Excess antibody covalently linked to a solid phase and directed against one end of the antigen is incubated with the sample and, after suitable washes, excess of a second, enzyme-labelled antibody directed against the other end is added. After further washes, the enzyme activity is determined. This provides a direct measure of the amount of antigen present and has been applied to the assay of both rat and human alpha foetoprotein (Maiolini & Masseyeff *J. Immun. Methods* **8,** 223; 1975).

A variation of the above is to add an excess of enzyme-labelled second antibody directed against the first. This has the merit that, for example, an enzyme-labelled goat anti-rabbit gamma globulin sera can be used for all assays based on rabbit first antibody.

In conclusion, it seems unlikely that the use of isotopically-labelled reactants will be replaced in the near future for assays requiring extreme precision or sensitivity in the pmol l^{-1} range. In our experience EIA has proved somewhat more complex, taken longer to perform and has been slightly less precise than RIA. However, EIA and related techniques have an important part to play in the developing world, in countries with severe restrictions on the use of radioisotopes; for such yes/no cases as the diagnosis of pregnancy or detection of Australia antigen (a marker of past or current infection with hepatitis) and, finally, for hapten assays in which their use avoids the need for a separation step. □

The puzzles of Hercules

from James Pringle

About a dozen pulsing X-ray stars have now been discovered. Among these perhaps the most intriguing, and certainly one that has stimulated much research and headache is the star Hercules X-1. This has an X-ray luminosity of around 10^{37} erg s^{-1} and the X radiation is pulsed with a period of 1.24 s. Almost as soon as this pulsing was discovered, the pulse period was found to vary in a sinusoidal manner with a period of 1.7 d. This, together with the fact that the X radiation from Hercules X-1 disappears for 9 h once every 1.7 d, indicated that the pulsing X-ray star was in orbit around a companion star. The sinusoidal variation of the pulse period is due to Doppler shift of the intrinsic pulse period as the source orbits its companion, and the regular disappearance of the flux is due to eclipses. These ideas were confirmed by the discovery that the companion star was the already known variable star HZ Herculis. The side of HZ Her facing the X-ray star is so intensely heated by the X radiation, that as the binary star system revolves the apparent optical brightness of HZ Her varies by a factor of four with a period of 1.7 d.

It is generally accepted that Hercules X-1 is a neutron star which has a very strong magnetic field. A field strength of around 10^{12} gauss, similar to those deduced for radio pulsars, is anticipated. The X-ray luminosity is produced by material, stripped off the surface layers of HZ Herculis, being accreted by the neutron star. A neutron star (radius about 10 km) is so compact that material falling onto it releases about 10–20% of its rest mass energy when it strikes the stellar surface. Thus a small trickle of material can give rise to a considerable luminosity. Material accreting onto the star is highly ionised and is therefore channelled along the stellar field lines down onto the magnetic polar caps. Thus the accretion energy is liberated in two hot spots, each about a kilometre in diameter, on the stellar surface. Such a high luminosity radiated from such a small area can only be emitted as X radiation. Then if the magnetic axis and the rotation axis of the star do not coincide, a stellar rotation period of 1.24 s ensures that the emitted radiation is pulsed at the correct period.

Although these basic ideas have been around for some time, the details of the radiation processes have proved too complicated for reliable predictions of pulse shapes and pulse spectra to be made. There are many uncertainties concerning the emission of radiation at the polar caps, in particular because the radiation emitted by the material striking the surface interacts with the column of accreting matter on its way out. It is also unclear exactly where the accreting material releases most of its energy, whether at the base of the accretion column at the stellar surface or more gradually as the column settles slowly supported by radiation pressure. The velocity of the material that is emitting or interacting with radiation is also important since infall velocities can be as high as half the speed of light and relativistic effects can come into play. In such a strong magnetic field the electron orbits perpendicular to the field direction are quantised with levels separated by an energy $13 B_{12}$ keV, where B_{12} is the field strength in units of 10^{12} gauss. Thus quantum calculations of the emission and absorption processes have to be made for the role of cyclotron radiation in such strong magnetic fields.

A further complication has been introduced by a recent suggestion that the beam pattern of X-ray emission may be produced not so much by anisotropic processes occurring near the stellar surface, but rather by a shell of material at the Alfvén radius or the surface of the magnetosphere (of radius about a thousand stellar

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radii) which has collected there before being channelled down to the magnetic poles. If the shell of material is not spherically symmetric—a likely event given the expected dipolar shape of the magnetosphere—then the variation in the amount of scattering or absorption of X rays in passing through the shell in the direction of Earth as the star and shell rotate can give rise to an apparent pulsed emission even if the neutron star produces a steady X-ray flux. In the absence of other information it was beginning to seem as if little progress could be made in the unravelling of these various effects.

Some recent observations, however, have given some cause for hope. At the Eighth Texas Conference held in Boston in December 1976 (see *News and Views* **265**, 402; 1977) J. Trümper announced evidence for a strong cyclotron emission line in the hard X-ray spectrum of Her X-1 in data obtained from a balloon-borne experiment. The apparent line is at 53 keV which if assumed to be cyclotron emission corresponds to a magnetic field strength of $4{\cdot}6\times10^{12}$ gauss. Because of background subtraction problems, this line although apparent in the raw spectral data, is more clearly visible when the data has been processed. The processing involves taking the spectrum of those photons received during the peak of the pulse, and subtracting the spectrum of the rest from it. In this way the background (assumed not to vary with a 1.24 s period) is removed. If the data is interpreted as for the most part a steady flux from the source, on which is superposed a fully modulated pulsed flux, then the spectrum obtained by this method is that of the pulsed flux. If this is not the correct interpretation then there are problems in deciding what the subtracted spectrum actually represents.

A paper by Coe *et al.* in this issue of *Nature* (page 508) reports confirmation of Trümper's results. The observations were made using the Ariel 5 satellite. Background subtraction is less of a problem with the satellite data, but even so the confirmed line seems to have shifted to 64 keV and the authors do not comment on the statistical significance of their result.

Nevertheless, the observations should provide a much needed clue to the X-ray emission processes occurring in Hercules X-1 and the other pulsed sources. Further observations—for example another balloon flight by Trümper's group this autumn with a larger area detector—are underway; thus not only do theorists have something to start thinking about but also the prospect, welcome or not, of a more rigorous test for any ideas they may come up with. ☐

Towards some enzyme mechanisms

from C. C. F. Blake

In the heady days of the late 1960s, when X-ray diffraction began to reveal the structures of enzymes in atomic detail, it seemed that the comprehensive view of the active site and the binding of substrate analogues that the technique provided, would lead rapidly to the establishment of a mechanism for each enzyme that was studied. In rapid succession proposals for the catalytic mechanisms of lysozyme, chymotrypsin and carboxypeptidase were put forward that contained profound insights into the chemistry practised by hydrolytic enzymes. However, since that time, as attention has become focused largely on the intracellular metabolic enzymes, the regular reports of the structures of new enzymes have not been accompanied by the expected mechanistic breakthroughs. Not altogether unexpectedly, it is beginning to look as though the non-hydrolytic enzymes are not going to yield the secrets of their activity as readily as the hydrolytic enzymes. This rather disappointing picture has recently been relieved to some degree by proposals on the catalytic mechanisms of two 'second generation' proteases.

The first protease, thermolysin, is a member of the family of neutral endopeptidases that require zinc for activity. Following their work on the structure of the enzyme (Matthews *et al. J. biol. Chem.* **249**, 8030; 1974) which revealed that its polypeptide chain was organised in quite a different manner from the other known zinc-requiring protease, the exopeptidase carboxypeptidase, Matthews and his colleagues have now proposed that despite this difference the mechanism of thermolysin is very similar to that of carboxypeptidase (Weaver *et al. J. molec. Biol.* **113**, 119; 1977). The mechanistic proposals are largely based on an examination of the binding of the potent inhibitor Phosphoramidon (N-(α-rhamnopyranosyl-oxyhydroxy-phosphinyl)-L-leucyl-L-tryptophan). Phosphoramidon is located in the active site of thermolysin with one of its phosphinyl oxygens located 2 Å from the essential zinc ion, completing its tetrahedral environment, while the other oxygen hydrogen bonds to the carboxylate of Glu 143. The leucine residue of the inhibitor is located in a hydrophobic pocket that appears to represent the specificity site of the enzyme, accounting for its known preference for bulky aromatic residues on the amino side of the scissile bond. A number of small conformational changes occur on binding but they are restricted to the active site region.

On the basis of the binding of Phosphoramidon, and some dipeptide inhibitors, Matthews concludes that the phosphinyl compound acts as an analogue of the presumed tetrahedral transition state intermediate of the enzymatic reaction. This has enabled a detailed model of the transition state of the true extended polypeptide substrate to be built into the active site, leading in turn to the proposed mechanism. The major features of the substrate binding are: the location of the carbonyl oxygen of the scissile bond as the fourth zinc ligand; the location of the large hydrophobic residue on the amino side of the scissile bond in the specificity site; and an extensive antiparallel β-sheet interaction between the polypeptide chains of the bound substrate and the enzyme. On either side of the susceptible bond are a carboxyl group (Glu 143) which, together with an associated water molecule, is 'buried' by the substrate, and His 231 that forms a hydrogen-bonded pair with Asp 226, rather like the charge relay in the serine proteases. The proposed mechanism indicates that Glu 143 promotes the nucleophilic attack of the buried water molecule on the carbon of the polarised carbonyl group of the substrate, forming a tetrahedral intermediate, and concerted with this step, or following it, His 231 donates a proton to the nitrogen of the scissile bond, leading to its collapse. This mechanism is very similar to one of the two alternative mechanisms proposed by Lipscomb for carboxypeptidase (Lipscomb *et al. Brookhaven Symp. Biol.* **21**, 24; 1968), except that proton donation is carried out by the histidine in the 'charge relay' rather than a tyrosine residue. By reverse analogy, it can be argued that the thermolysin mechanism supports the proposal that Glu 270 promotes the attack of a water molecule on the substrate carbonyl in the carboxypeptidase mechanism, against the alternative of direct nucleophilic attack by the glutamic acid.

Precisely the same argument is used in the case of the second protease, penicillopepsin. The three-dimensional structure and amino acid sequence of this enzyme were reported recently (Hsu *et al. Nature* **266**, 140; 1977), clearly demonstrating it to be a member of the acid proteases; the last major family of proteases to be examined by X-ray diffraction. Now James and his

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colleagues (James *et al. Nature* **267**, 808; 1977) have immediately followed the description of the structure with a proposal for the enzyme's mechanism —just like the old days! This proposal is based largely on the remarkable environment of the two essential acid groups, Asp 32 and Asp 215, although binding studies have been carried out on the potent inhibitor 1,2,epoxy-3-(p-nitrophenoxy) propane (EPNP). The difference map of inhibitor binding shows that the two molecules of EPNP have reacted covalently with penicillopepsin, one with the carboxyl group of Asp 32, the other with the carboxyl group of Asp 215. Associated with inhibitor binding is clear evidence of a conformational change to Tyr 75 that brings it into the active site.

The key to the mechanism of the acid proteases seems to reside in the environments of the two acid groups. Asp 32 is located in an unusually tight hydrogen bonding net, that involves its carboxyl group in interactions with (1) the carboxyl of Asp 215, (2) the hydroxyl of Ser 35, (3) the main-chain NH of Gly 217, (4) a bound water molecule, and (5) the main chain atoms of the 32–33 and 33–34 peptide groups. This complex of interactions makes Asp 32 inaccessible for the formation of covalent interactions with substrates without conformational changes in the active site, which its particular structure seems to rule out. Asp 215 is on the other hand rather more accessible, but since it seems established that this group has the higher *p*Ka, it is unlikely to be the attacking nucleophile.

James points out that the resolution of this problem may be sought in the cluster of polar and ionic side chains that lie in the hydrophobic core near the two acid groups. These include Asp 304, which makes hydrogen bond contact with the main chain carbonyl of Thr 216 (the same peptide whose NH hydrogen bonds to Asp 32), nearby is an ion pair, Lys 308 and Asp 11, which has as neighbours a buried tyrosine, residue 14, and a surface histidine, residue 57. With the exception of the histidine, all these residues are conserved in porcine pepsin. This cluster of buried residues is interpreted as an extended charge relay system. It is assumed that the binding of substrate in the large cleft of the enzyme will affect two associated hairpin loops carrying Asp 11 and Tyr 75, causing a minor conformational change to the former and a larger one to the latter, similar to that observed when EPNP reacts with the two acids, bringing Tyr 75 into the active site. The movement of Asp 11 would disrupt its ion pair interaction with Lys 308. A series of changes then ensues that involves Lys 308 pairing with Asp 304 and the protonation of the 216–217 peptide bond that would enhance the electrophilicity of the proton shared between Asp 32 and Asp 215 This proton could then function to polarise the carbonyl group of the substrate in a mechanism that is otherwise identical to the zinc-carbonyl mechanism of carboxypeptidase. Asp 32, the analogue of Glu 270 in carboxypeptidase, would promote the attack of its bound water molecule on the carbon atom of the polarised carbonyl group of the substrate, while Tyr 75, the analogue of Tyr 248 in carboxypeptidase, protonates the nitrogen of the scissile bond. Thus in James's view the mechanism of the acid proteases is essentially the same as that of carboxypeptidase, with modifications to make it competent at a low *p*H. This means replacing the Lewis acid, Zn^{2+}, with a proton whose electrophilicity is enhanced by the charge relay involving the protonated peptide, and the placing of Asp 32 in an unusual environment to keep it in the ionised state at very low *p*H.

Although the mechanism of the acid proteases proposed by James has some controversial features, not least the involvement of a peptide bond, the remarkable environment of the essential acid groups will provide a solid basis for any further discussion, and this is what the X-ray crystallography of enzymyes should be all about. ☐

Visiting Halley's Comet

from David W. Hughes

COMETARY missions have been discussed in varying degrees of detail on both sides of the Atlantic for well over a decade; spacecraft have been chosen, experiments have been planned, data and science returns estimated, impact hazards evaluated. All this as yet to no avail. Why? Comet trips are not cheap; $100,000,000 is a reasonable bargain-basement estimate. Also they represent a distinct new start in space exploration, needing new people and new ideas and the old teams of lunar, planetary and magnetospheric space scientists seem only too willing to plump conservatively for well tried experiments or at best minor advances in techniques. Also when the budget for space exploration is limited, the 'jobs for the boys' and 'let's play safe' syndromes come to the fore.

Comets are also minor objects in the Solar System. They are studied by a keen but small band of scientists. Very few of them get into the news, comet Kohoutek being the only one to make a stir recently and this disappointed most tax paying (and consequently space exploration supporting) people by being much fainter than originally predicted. The missions are also difficult; comets, being small, are hard to find and considerable launch and trajectory correction accuracy is required to get close to them. Ballistic transfer usually gives a fly-by speed of upwards of 7 km s^{-1}. Measuring the properties of a cometary coma and nucleus when you are flying by at 25,000 km h^{-1} is not easy and also the time during which cometary phenomena are being investigated makes up a very small percentage of the total mission time (however the same could easily have been said of the first Mariner trips to Mars and Ranger trips to the Moon).

How does comet Halley change all this? First, it has the advantage of being famous. Second, it has been around a long time. It passes perihelion about every 76 yr, is unusually bright and seldom goes unnoticed. Observations go back as far as 239 BC when it was mentioned in ancient Chinese records and it has been seen 28 times since. Third, it is rather awkward to get to, its retrograde orbit giving a ballistically transferred spacecraft a fly-by speed of almost 60 km s^{-1}. Halley is therefore an ideal candidate for a rendezvous mission, but this would require considerable expenditure of power. Ion drive and solar sail propulsion have been suggested as ways round this problem.

These two mechanisms are still at the theoretical stage but have caught the imagination of space technologists to such an extent that Halley's comet is being actively suggested as a first mission application for the two competing techniques. Scientifically, Halley's comet is as good a start as any to a programme of cometary investigation. The first mission must be regarded as the precursor to a series of investigations which will eventually take in comets of diverse qualities and build up a complete picture of the family. The exact form of the cometary nucleus is still a mystery and there is debate as to whether it is a condensed, dirty, icy snowball or a large swarm of smaller particles. Comets decay to produce meteor streams and so feed the zodiacal dust

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cloud. Cometary chemistry is very similar to that of interstellar gas but fortunately comets are closer. The interaction between the plasma in the cometary coma and the high speed magnetoplasma of the solar wind poses exciting physical problems. Comets are also probably in the form of the original condensate from the pre-Solar System nebula and as such furnish an outstanding clue in cosmogony.

Halley's comet was visible to the naked eye over a four month period at its 1910 apparition. The nuclear region was several thousand kilometres in diameter but on 18 May, 1910, when Halley transited the Sun, no solid nucleus was observed against the solar disk indicating that, if this existed, it must be less than 50 km across. The coma was about 3×10^5 km in diameter during the postperihelion phase, and had a continuous background spectrum with superimposed CN and C_2 lines. Transient phenomena were observed in the inner coma regions. Halley is a very dusty comet and also has non-gravitational effects acting on its nucleus leading to a lengthening of the orbital period by about 4.1 d per apparition.

The main scientific objectives of the fly-by are to image the nucleus, to find its size, shape, rotation period and colour, to determine the nature of the multiple condensations seen in 1910, to determine the abundance and spatial distribution of neutral molecules, radicals and charged particles in the coma and tail, to investigate the shockwave interaction between the cometary plasma and the solar wind, to measure the spatial density and size distribution of the emitted dust particles and to investigate the time variation of the coma's structure and brightness.

Opportunities for ballistic missions to Halley have been studied by Robert W. Farquhar and William H. Wooden II (NASA Goddard Space Flight Centre) and have been recently reported in NASA Publication No. X-580-77-46. At a fly-by speed of $60\ \mathrm{km\ s^{-1}}$ the spacecraft will pass through the inner and outer coma in 14 and 18 min respectively. The miss distance from the nucleus would be about 2,000 km. The spacecraft could approach closer to the nucleus with onboard navigation but previous visual observations indicate that Halley has multiple nuclear condensations and it would be difficult to know which one to pick when the spacecraft is a considerable distance from the comet. At perihelion Halley is about 0.59 a.u. from the Sun. Perihelion passage occurs on 10 February, 1986 and most investigating spacecraft plan to visit Halley within a period of about 60 d either side of this date.

Rendezvous with Halley

Farquhar and Wooden put forward four possible missions. The first is a dual launch around 4 July, 1958 of the spacecraft using a single Space Shuttle, one planned to intercept Halley 63 d before perihelion (at 1.37 a.u.) the other 39 d after perihelion (at 1.00 a.u.). One approaches Halley from the sunward side the other from the anti-Sun direction. The second senario again uses the Shuttle to launch two spacecraft, this time on 10 March, 1985. One intercepts Halley 58 d before perihelion passage, the other flies off to intercept two other comets, Giocobini-Zinner on 11 September, 1985 and Borelly on 25 December, 1987 these interceptions taking place at 1.03 and and 1.36 a.u. respectively. The third launches the spacecrafts at Halley in a trajectory that returns to Earth after passing the comet. They then swing past the Earth and are retargeted on Borelly to fly-by in January, 1988 or comet Tempel-2 in September, 1988. The final proposed mission has a dual launch in August, 1985, with both spacecraft encountering Halley at the same time, one passing the nucleus on the sunward side at a distance of about 2,000 km and the other moving through the tail about 30,000 km behind the nucleus.

The Warsaw-Moscow soil structure project

from I. J. Smalley

A JOINT programme to provide a set of soil structure standards for use in engineering geology and soil mechanics has been under way for five years at the Universities of Warsaw and Moscow and is now nearing completion. The first applications of the scanning electron microscope (SEM) to soil structure studies were made in the late 1960s and in 1971 a project was initiated by B. Grabowska-Olszewska of the Warsaw University Engineering Geology Institute and V. Osipov and V. Sokolov of Moscow University with the aim of providing a complete range of reference structures from clay sediments of different ages and from different sedimentary environments.

The scanning electron microscope has not proved as effective a tool in the investigation of soil structures as was hoped when studies began and much of the earlier enthusiasm is now waning. There is, however, a growing emphasis in engineering geology and soil mechanics on the study of earth materials in some detail and Attewell and Farmer (*Principles of Engineering Geology*, Arnold, 1976) and J. K. Mitchell (*Fundamentals of Soil Behaviour*, Wiley, 1976) in their new books both present extensive sets of soil microstructures, but they are essentially illustrations; the relating of soil structure to engineering properties has not been achieved. A set of standard structures with related properties may help in the making of this necessary connection.

The Warsaw-Moscow project has been hampered by the lack of SEM facilities in Poland and the USSR and most of the structural photographs were obtained using the American built 'Cwik-Scan' 106A SEM of Moscow University. Samples were taken from Poland and the European USSR and all geological areas are represented from Proterozoic to Holocene. The programme involved the examination and testing of 86 samples of eluvial, hydrothermal, marine, lacustrine, alluvial, glacial and glacial-lacustrine origins. Five structural elements were identified: primary clay particles, micro-aggregates, aggregates, grains and inclusions of microfauna, microflora and microcrystals of salts and ore minerals. The structural data, usually presented as 5000× and 500× photographs, is supplemented by a range of property measurements including specific gravity, water content, density of wet soil, porosity, specific surface, liquid limit, plastic limit, uni-axial and pure shear strengths and it is the relation of this set of mechanical data to the structural representations which makes the project so valuable.

E. M. Sergeev (Moscow) and W. C. Kowalsky (Warsaw) have editorial attachment and various western investigators have been involved; in particular David Krinsley of Arizona State University, doyen of the SEM sand gazers, has recently been in Warsaw helping with the final stages of the project; and R. Pusch of Lulea University has been giving encouragement. The 1973 Gothenburg Engineering Soil Structure Symposium organised by Pusch revealed the disorder and confusion in the field; perhaps the Warsaw-Moscow project will provide a necessary foundation for future studies.

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All these ballistic missions would produce an enormous amount of fascinating new data concerning comets and they have the advantage of a late launch date so that the orbital parameter of the comet can be updated by observations taken as it comes towards the Sun. They also have a short transit time, the spacecraft usually encountering the comet about 8 months after launch. Launch energies are also relatively low, C_3 being around 12 km^2 s^{-2} for the missions that do not have an Earth return and about four to five times this for the one that does. One disadvantage is the high fly-by speed, most spacecraft encountering Halley nearly head on

Solar sails and ion drive

Two rather unorthodox schemes have been proposed recently to get over this problem Both require advanced propulsion capabilities and these capabilities have been little developed to date. Both require flight times around 3 to 4 yr and thus need launch dates in early 1982. Both schemes have the enormous advantage of enabling the spacecraft to rendezvous with the comet and move off with Halley on its journey away from the Sun.

J. L. Wright (Jet Propulsion Laboratories, California) and J. M. Warmke (Battelle Columbus Laboratories, Ohio) discuss the solar sail concept in the *American Institute of Aeronautics and Astronautics* paper number 76–808. Here a sail, between 300 and 800 m across, would be attached to an existing spacecraft, like Mariner or Pioneer, the propulsion force being derived from the reflection of solar photons About 0.9×10^{-5} N m^{-2} could be obtained at 1 a.u. By tilting the sail with respect to the Sun's direction the spacecraft could tack on an inward or outward trajectory. No additional propellant is required; one literally sails through space. At 1 a.u. a typical spacecraft could be accelerated at between 0.2 and 1.3 mm s^{-2}, corresponding to a mass loading on the sail of between 40 and 60 g m^{-2} Wright and Warmke suggest that with considerable development effort the sail could be ready for a first mission application in late 1981. 640,000 m^2 of sail could accelerate a 800 kg spacecraft to Halley. Launch would be around January, 1982 the vehicle first spiralling inward to about 0.3 a.u then being cranked into an orbit of inclination 162° Gaining energy it would move out to about 2 a.u. before returning to the inner Solar System nearly along Halley's orbit, rendezvousing with the comet after perihelion somewhere between 1 and 2 a u from the Sun

The second scheme uses an advanced form of solar electron propulsion and has been put forward by K. L. Atkins (Jet Propulsion Laboratories) and C. Terwilliger (Boeing Areospaces Co) in *AIAA Paper* 76—1069. They note the massive advance in electron propulsion performance. 1973–74 vintage required 50 kg of spacecraft systems to produce a kilowatt of power. New 3-mm thick solar cells and high powered, direct-drive ion engines have reduced the specific mass to 10 kg giving a fivefold increase in power to weight. To get to Halley the spacecraft would be launched around April, 1982 in an outbound flight towards Jupiter. The ion drive vehicle remains outside Halley's flight path, makes a powered 'U-turn' at about 4.5 a.u and speeds back towards the Sun essentially already in Halley's orbit, simply waiting for Halley to overtake. Flight time is around 3.75 yr, rendezvous occuring about 50 d before perihelion and maximum cometary activity. The spacecraft then stays with Halley having in Atkins and Terwilliger's words "a pre-performance seat to watch the whole show"

Halley is even now coming in towards the Sun and has already passed the orbit of Uranus. It is hoped that we can be adventurous enough, both scientifically and financially, to get one of the schemes outlined above ready to meet it as it comes by in 1985–86· if not we will have to sit back until 2026. □

Environmental mutagenesis

from Margaret Fox

The 2nd International Conference on Environmental Mutagenesis was held at Edinburgh on 10-16 July, 1977.

A CENTRAL problem in the field of environmental mutagenesis is to design 'short-term' tests, using either prokaryotic or eukaryotic systems, or both, capable of detecting many different types of genetic damage. During the conference the deficiencies and advantages of the currently available tests were extensively discussed.

Carcinogens in most instances have produced positive results in screening programmes for mutagens in contrast to non-carcinogens (B. N. Ames *et al.*, University of California, Berkeley) and it seems likely that DNA damage is intimately involved in both cases. However, there are some notable exceptions to this rule. M. S. Legator (Department of Preventative Medicine, University of Texas) in discussing microbial activation systems such as Ames's *Salmonella* test, pointed out that not only do such systems fail to detect certain types of genetic damage, for example, chromosome non-disjunction, they also fail to detect several other classes of known mutagen and carcinogen such as ionising radiation, chlorinated pesticides, azo dyes and certain metal carcinogens.

The necessity for metabolic activation of many carcinogens is now well established, and S-9 preparations of rat liver microsomes are now routinely included in many screening tests. The almost exclusive use of liver preparations and the assumption that the inclusion of liver microsomes in microbial systems will accurately mimic *in vivo* mammalian metabolism was criticised.

Clear evidence for a difference between bound products in mammalian and prokaryotic DNA was presented by A. Dipple and coworkers (Frederick Cancer Research Centre, Maryland). Incubation of ^{3}H-7-12-dimethyl benz[a]anthracene (^{3}H-DMBA) with cultured mouse embryo cells resulted in at least three identifiable DNA reaction products whereas only one of the three was identifiable when two of Ames's *Salmonella* strains were incubated with ^{3}H-DMBA in the presence of liver microsomes. Because of such deficiencies in prokaryotic test systems, increasing attention is now being paid to eukaryotic and in particular to mammalian systems.

For risk estimates it is important to have precise knowledge of dose–response relationships, and a direct approach to this has been made in several laboratories by the use of cultured human fibroblasts. V. Mayer (Michigan State University) presented data indicating a threshold or no-effect dose for mutation to 8-azaguanine resistance after exposure of human fibroblasts to ultraviolet and some reactive hydrocarbon derivatives. The implications of these observations are of such importance that it is essential they be validated in other laboratories and for other loci, particularly in view of the fact that alterations in methodology can profoundly alter the shape of the dose–response curve for mutation to 8-azaguanine resistance in V79 Chinese hamster cells (S. McMillan, Paterson Laboratories, Manchester) Linear dose–response curves with no threshold have been found for several mutagens tested at both the HGPRT and TK locus in mouse L5178Y cells (A. Knapp, Department of Radiation Genetics and Chemical Mutagenesis, Leiden) and for 8-methyoxypsoralen plus near ultraviolet light tested at the

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HGPRT locus in V79 cells and human diploid fibroblasts (J. Simons, Department of Radiation Genetics and Chemical Mutagenesis, Leiden).

8-methyoxypsoralen (8-MOP) plus long wave ultraviolet light is an effective treatment for psoriasis, a debilitating skin disease in man. 8-MOP in the dark is an effective frameshift mutagen in *Escherichia coli* and *S. typhimurium* (B. Bridges, MRC Cell Mutation Unit, University of Sussex). In contrast, base pair substitution mutagenesis occurs after exposure to 8-MOP plus near ultraviolet light. 8-MOP plus near ultraviolet light is mutagenic in both V79 cells and human diploid fibroblasts when assayed at the HGPRT locus (Simons), but no ouabain-resistant mutants could be recovered in V29 cells (C. Arlett, MRC Cell Mutation Unit, Sussex). 8-MOP in the dark does not seem to increase the frequency of sister chromatid exchanges in human lymphocytes *in vitro* although 8-MOP plus near-ultraviolet had a positive effect (A. Brøgger, Norsk Hydro Institute, Oslo). Many assumptions have to be made to estimate risk from such data and essentially negative results for induced chromosome aberrations in lymphocytes of psoriasis patients undergoing treatment have been reported by Brøgger. However, the treatment should be regarded as potentially hazardous although the benefit to individual patients may well offset the overall risk.

Several presentations discussed completely *in vivo* 'short-term' methods for assay of induced somatic mutation in mammals. Mice heterozygous for the *dilute* and *leaden* loci irradiated late in foetal life show patches of abnormal pigment cells when whole mounts of epidermis are made shortly after birth (A. G. Searle, MRC Radiobiology Unit, Harwell). Such changes, the result of recessive mutations at the two coat colour loci, are observable at the cellular level as alterations in the shape of and pigment density in melanocyte precursor cells.

Possible pitfalls in the use of this method were discussed by L. B. Russel (Oak Ridge, Tenessee) who emphasised the need for microscopic scoring, as coat colour spotting on a macroscopic level can be the result of other nonspecific effects of mutagen exposure such as decrease in number of precursor cells and/or disturbances in differentiation. With careful attention to such problems, induced mutant frequencies for a number of test compounds roughly paralleled but were significantly higher than those found for germ cell mutations (specific locus test), suggesting that possibly different types of genetic damage are being assayed.

In vivo exposure of animals to mutagens followed by *in vitro* assay is relatively rapid, organ and tissue-specific effects can potentially be monitored, and the approach has the advantage that injected compounds are subjected to full metabolism and detoxification. Using this approach, B. Dean (Shell Research, Sittingbourne) demonstrated increased frequencies of 8-azaguanine and ouabain-resistant clones in cultured cells derived from different tissues of 3-week-old Chinese hamsters previously treated with ethyl methanesulphonate, methyl nitrosourea and diethylnitrosamine.

In vivo exposure and *in vitro* detection is the principle on which monitoring of exposed human populations by scoring chromosome aberrations in lymphocytes is based. For accurate assessment of induced damage it is important that aberrations be scored in the *first* metaphase after phytohaemagglutinin stimulation. It is now possible to identify first metaphase exactly by culturing lymphocytes in BUdR and identifying second divisions as showing differential staining of chromatids. Using this method, Grossen (Christchurch Hospital, New Zealand) demonstrated that by 48 h, the usual sampling time used, all cultures of lymphocytes examined showed second divisions (range 2–53%). The scoring of such a heterogeneous population could lead to erroneous conclusions with respect to overall sensitivity.

The *in vivo/in vitro* approach was also adopted by R. Albertini (University of Vermont Medical College). This method of mutagen detection, based on autoradiographic measurements of the frequency of phytohaemagglutinin-stimulated lymphocytes which continue to incorporate ^{3}H-thymidine in the presence of 6-thioguanine (6-TG), was validated by the demonstration of a high frequency of such cells in patients suffering from Lesh-Nyhan disease and known to lack HGPRT activity. Significantly higher frequencies of 6-TG resistant lymphocytes were demonstrated in patients receiving cancer chemotherapy. The molecular basis of the 6-TG resistance in this system is at present unknown, but the authors invoke somatic mutation and suggest that the assay could be used in parallel with cytogenetic screening for monitoring genetic damage.

Overall the general feeling was that as yet no single test or panel of tests can accurately predict potential hazards to man, and that 'short-term' tests, particularly in microorganisms, although offering the possibility of rejecting dangerous chemicals at an earlier stage cannot yet completely replace conventional carcinogenic tests with animals.

The concern of industry and the scientific community in this field is indicated by the recent formation of the International Commission for Protection against Environmental Mutagens and Carcinogens (ICPEMC) under the Chairmanship of Professor S. Sobels (Leiden) to act as an advisory body and collate data on an international level. In this way it is hoped that progress can be made in protecting man from his hazardous environment.

Recombination at Nethybridge

from a Correspondent

The fourth bi-annual EMBO Workshop on the Molecular Basis of Genetic Recombination was held at Nethybridge, Scotland, on 20-25 June, 1977. As on previous occasions, a deliberate attempt was made by the organisers to bring together scientists working on a very wide range of recombination systems.

An essential intermediate in recombination is hybrid or heteroduplex DNA, which may alter its length by branch migration. This involves the breakage and formation of hydrogen bonds between complementary bases at the point where polynucleotide chains exchange pairing partners. For the first time, exact measurements of the rate of branch migration *in vitro* have been made by R. Warner (University of California, Irvine) and C. Radding (Yale University) using φX174 or G4 phage recombination intermediates. The rate of branch migration seems too low to explain the spontaneous formation of long stretches of hybrid DNA *in vivo* and it is therefore likely that the process is driven by enzymes or DNA binding proteins. Electron microscope studies of recombination intermediates in phage PMB-9 by D. Dressler (Harvard University) demonstrate the formation of reciprocal hybrid DNA in molecules held together by a symmetrical strand exchange point. This is dependent on the *rec*A function of *Escherichia coli,* but independent of *rec*B.

Predictions of the Aviemore model of genetic recombination (proposed by M. Meselson and C. Radding at the second EMBO Recombination Workshop in 1973) were confirmed by experiments in *Ascobolus* reported by J-L. Rossignol (Université de Paris, Orsay). The model states that recombination is initiated by an asymmetric strand transfer followed by rearrangement, or isomerisation, of polynucleotide chains to give hybrid DNA on two chromatids. The location of hybrid DNA can in part be determined by examining the pattern of gene conversion and post-meiotic segregation (PMS) of individual markers in meiotic octads. Examination of mutants at increasing distances from the point where recombination is initiated, shows that there is an increase in the proportion of aberrant 4 : 4 octads, which have two PMS events and can only arise from reciprocal hybrid DNA. Furthermore, among a class of mutants that show unequal numbers of 6 : 2 and 2 : 6 segregations (gene conversions), the disparity increases with distance from the initiation point of recombination, and Rossignol was able to show that this is expected if hybrid DNA is initially asymmetric, but is later symmetric. In studies on the correction of mismatched bases in hybrid DNA, P. Pukkila (National Institute for Medical Research, Mill Hill) constructed artificial heteroduplex molecules from φ80 phages containing mutant or wild type tRNA^{Tyr} genes of *E. coli.* Since the tRNA sequences are known, it is possible to construct molecules with known base mismatches. The enzyme DNase 1 from *Ustilago,* which is implicated in gene conversion, cleaves these molecules at or near the mismatch, but surprisingly, purine/pyrimidine mismatches can be a better substrate than other mismatches. In collaborative studies with M. Meselson (Harvard University), M. Radman (Université Libre, Brussels) reported experiments which may explain the biological function of the mismatch repair system. λ phage heteroduplexes were constructed in which one strand was methylated and the other was not. The *in vivo* correction system appears to remove the mismatched base in the non-methylated strand. The significance of this observation is that newly replicated DNA is non-methylated and therefore the errors in DNA synthesis will be preferentially corrected to wild type. PMS is due to the failure to correct mismatches in hybrid DNA, and R. Holliday (National Institute for Medical Research, Mill Hill) described a new method in *Ustilago* for detecting PMS without tetrad analysis, which may facilitate the search for mutants lacking this particular repair function.

The pairing of chromosomes at meiosis is mediated by an organelle known as the synaptonemal complex (SC). A. Carpenter (University of California, San Diego) described the accumulating evidence in *Drosophila* that electron-dense nodules on the SC are the sites of crossing over. Meiotic mutants with altered frequency or distribution of cross overs have corresponding changes in nodule formation. In the silkmoth *Bombyx,* S. Rassmussen (Carlsberg Laboratory, Copenhagen) reported that nodules are absent from the SC of females where there is no crossing over, but present in males which do recombine chromosomes.

Studies in bacteria of insertion sequences (IS), transposons and phage mu have opened up a new field of *rec*A independent recombination events that do not require significant sequence homology. However, the sequence at the ends of a given element (which are often inverted repeats) is essential for the recombinational event promoted by this element. Recent results with IS (J. Besemer, University of Cologne) transposons (N. Kleckner, Harvard University) and phage mu (A. Bukhari, Cold Spring Harbor, New York, and P. Van de Putte, University of Leiden) have shown that in all these systems excision of the element is usually associated with the formation of deletions or substitutions. IS and transposons seem to have preferred regions of integration although there are a number of insertion sites within a given region. It was previously known that integration into host chromosomes is an essential step in the lytic cycle of phage mu, but new isotope experiments indicate that daughter molecules rather than the parental phage genome become integrated. Similarly on induction of a mu lysogen most of the original prophage remains in the chromosome, the daughter molecules being replicated from that template and then reintegrated elsewhere.

The biochemical basis of site-specific *int* mediated recombination has been established by the *in vitro* system of H. Nash described by L. Enquist (both of the National Institutes of Health, Bethesda). A λ circle containing two separated attachment sites, which has been supercoiled by DNA gyrase, is a substrate for reciprocal recombination mediated by the purified *int* protein. Enquist's own results indicate that short regions of hybrid DNA are formed during this type of recombination *in vivo* and that these can migrate from the attachment site if the adjacent region is homologous; in other words, *int* recombination is not absolutely site specific. The basic problem in recombination mediated repair is the mechanism whereby damaged DNA can seek out an undamaged homologous molecule. P. Howard-Flanders (Yale University) now has evidence for a 'transendonuclease' activity in *E. coli,* which is *rec*A dependent. In response to psoralen-damaged phage DNA, the enzyme introduces nicks into supercoiled homologous, but not heterologous DNA *in vivo,* as well as in an *in vitro* system. K. Powell (University of Newcastle-on-Tyne) reported that the *rec*A product in *E. coli* is protein X and that the *tif* mutant (which maps in the *rec*A gene) produces an X protein with altered electrophoretic mobility in a two dimensional gel system.

Chi mutants in bacteriophage λ are hotspots for recombination, mediated by the bacterial *rec* system. F. Stahl (University of Oregon, Eugene) has now examined λ genomes in which sequences of bacterial DNA have been inserted. Some of these contain naturally occurring *chi* sequences, whereas others do not. He estimates that there is one recombination hotspot for every ten or so bacterial genes. Mutants with properties similar to *chi* are also known in *Neurospora* and *Schizosaccharomyces,* and similar sequences probably occur naturally since it is known that recombination in fungi is distributed unevenly along the chromosome. Moreover, P. Thuriaux (University of Bern, Switzerland) came to the conclusion that recombination may be restricted to structural genes in higher eukaryotes, since neither total map distance of the genome nor the length of individual structural genes varies with DNA content. It is evident that the text-book dogma that recombination is distributed randomly along chromosomes is about to be finally dispelled, both in prokaryotic and eukaryotic organisms. □

A hundred years ago

The Dutch Geographical Society has received a report from the expedition recently sent out to explore Sumatra. One division left Padary in the middle of May for the mountainous centre of the island. They have successfully penetrated into these hitherto unknown regions, and describe them as of surpassing grandeur. The mountain sides are clothed to the very top with a most luxuriant forest growth, almost impenetrable to the sun's rays. The inhabitants consist of a few utterly degraded Malays gathered together in wretched villages. The health of the expedition is excellent.

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review article

Interstellar molecules

B. Zuckerman*

Recently discovered interstellar molecules are a powerful new tool in the arsenal of the astrophysicist. Operating in a completely non-terrestrial environment, interstellar chemistry produces both everyday molecules likely to be found around the house and exotic species never before observed on the Earth. Interpretation of the molecular spectra requires an interdisciplinary approach.

MOST of the mass of our Milky Way Galaxy is contained in the stars. There is also a very cold and tenuous gas between these stars that comprises a small percentage of the total mass of the Milky Way. While not as visually spectacular as the stars and the bright emission nebulae this cold gas has, nonetheless, proved to be an exceptionally powerful tool for the study of such diverse astrophysical problems as cosmology, cosmogony, galactic structure, stellar evolution, star formation and nucleosynthesis. The cold gas, which is composed of atoms, molecules, and 'dust' grains (of uncertain composition), is most effectively studied by means of radio astronomical spectroscopy. Except for a few diatomic molecules, all of the 40-odd known interstellar molecules have been detected only by means of their radio spectra (Table 1). It seems that production, destruction and excitation of these molecules take place in a hostile (by terrestrial standards) environment by means of various processes involving atoms, ions, free radicals, molecular ions and neutral molecules, solid surfaces, cosmic rays and high and low energy radiation. With but one exception the radio spectra of all of the molecules in Table 1 have been detected within the past eight years. Data have accumulated extremely rapidly and the promise for continued rapid growth of the field is bright if large new antennae and interferometers sensitive to millimeter wavelength radiation are constructed. This fluid situation renders a definitive review impossible at this time. I will, here, describe only a few of the more interesting recent developments: those that are basically astronomical as well as those that involve close collaborative efforts among astronomers, physicists and chemists.

Interdisciplinary approach

The most important contribution of the laboratory spectroscopist to the radio astronomer is the measurement and assignment of molecular transition frequencies. Because most of the molecules in Table 1 are stable and have high vapour pressures at room temperatures, it is relatively easy to measure and assign their complete rotational spectra. The astronomer simply uses this type of information to search for and identify interstellar molecules. There are, however, molecules such as free radicals and molecular ions that cannot easily be studied in the laboratory because they are very unstable in laboratory conditions, and others that have very low vapour pressures at room temperatures. Many such molecules have been discovered by accident by radio astronomers who were searching for other molecules with known spectra. Even in the absence of any laboratory data tentative identification is often possible. Considerations of cosmic abundances of atoms, interstellar chemistry, calculations *ab initio* of molecular structures, isotopic substitution, and hyperfine structure when combined together are often sufficient to identify a molecule with considerable reliability. In this way interstellar HCO^+, N_2H^+ and HNC were all correctly identified before they were only very recently observed in the laboratory with new experimental techniques[1–5]. The international flavour of the field is highlighted by the nearly simultaneous observations of HNC in laboratories in the USA, West Germany and Australia[3–5]. The definite confirmation of the existence of HCO^+ and N_2H^+ in considerable abundances in interstellar molecular clouds show that ion–molecular reactions[6–7] must have an important, possibly the dominant, part in interstellar chemistry.

Because of the low kinetic temperatures (10–100 K) and gas densities (10^2-10^6 cm^{-3}) in most molecular clouds conditions are generally not those of thermodynamic equilibrium. The detailed population distribution in the rotational ladders of interstellar molecules can, in principle, be used to deduce the physical conditions in the clouds (especially the temperatures and densities of both neutral and ionised species). In practice one assumes that, at least microscopically, conditions are in steady state and collisional and radiative rates into and out of the various rotational levels are in balance. Radiative transition probabilities are generally well known. The most abundant constituents of molecular clouds are hydrogen molecules and helium atoms. Neither has a spectrum accessible to radio astronomy, but they play the dominant part in the collisional excitation of the less abundant polar molecules listed in Table 1. Unfortunately, little has been known about the magnitude of and selection rules for collisional transitions under low temperature interstellar conditions.

Recent quantum mechanical calculations[8–10] of collisional rates between He and HCN, CO and N_2H^+ have removed much of the uncertainty in this area. The propensity rules for the collisional transitions differ from the radiative selection rules and indicate differences between neutral–neutral and neutral–ion collisions as well. This allows the possibility of highly non-equilibrium population distributions, namely inversion (heating) and anti-inversion (cooling), in some rotational transitions. Sometimes this can lead to exotic phenomena such as the high gain SiO, H_2O and OH masers discussed below. Because of the very general competition between ordinary spontaneous radiation, and the simple collisions mentioned above, interesting phenomena can occur even over most of the volume of common molecular clouds.

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Chemical history of the galaxy

Among the many astrophysical problems that can be investigated with interstellar molecules is the galactic history of the elements[11]. Stars return a significant percentage of their mass back to the interstellar gas out of which they were born. Red giants and even more evolved stars, such as planetary nebulae and supernovae, are very important participants in this interchanges of mass. The 'cooking' of hydrogen and helium into heavier elements such as carbon, oxygen, and nitrogen deep in the interior of giant stars and the subsequent expulsion of these elements back into interstellar space determines the chemical evolution of our Galaxy. Since it is believed that originally the Universe was composed almost entirely of hydrogen and helium, the formation of planets like the Earth and their living inhabitants is entirely dependent on this mass exchange between stars and gas.

Recent studies of relative isotopic abundances in various interstellar molecules, such as H_2CO, CO, HCO^+, HCN and CS[12-16], promise to help us unravel the long history of our galaxy. It seems, for example, that there may have been some enrichment of ^{13}C relative to ^{12}C and of ^{17}O relative to ^{16}O in the interstellar medium since the time our Solar System formed some 4.6×10^9yr ago. With the aid of theoretical calculations on stellar interiors, these observations may tell us about the types of stars that are responsible for the heavy elements found in the interstellar gas. For example, the apparent difference in the $^{15}N/^{14}N$ ratio between the central and outer portions of our Galaxy has been interpreted[15] to imply a greater proportion of low mass stars and a lesser proportion of supernovae near the galactic centre.

Unfortunately, a good deal of the interpretation is still beclouded by the possibility that isotopic fractionation effects are important in the formation and/or destruction of interstellar molecules[17]. Therefore, it may not be possible

Table 1 Known interstellar molecules as of January 1977

Number of atoms	Molecule	Optical/UV	Radio spectrum	Detected in astronomical objects*
2	H_2—hydrogen	✓	—	1, 2(?), 3
	OH—hydroxyl radical	✓	Λ-doubling	1, 2, 3, 4
	SiO—silicon monoxide		Rotational	2, 3
	SO—sulphur monoxide		Rotational	3
	SiS—silicon monosulphide		Rotational	2
	NS—nitrogen sulphide		Rotational	3
	CH^+—methylidyne ion	✓	—	1
	CH—methylidyne	✓	Λ-doubling	1, 3
	CN—cyanogen radical	✓	Rotational	1, 2, 3
	CO—carbon monoxide	✓	Rotational	1, 2, 3, 4
	CS—carbon monosulphide		Rotational	1, 2, 3
3	H_2O—water		Rotational	2, 3, 4
	H_2S—hydrogen sulphide		Rotational	3
	SO_2—sulphur dioxide		Rotational	3
	HCN—hydrogen cyanide		Rotational	1(?), 2, 3
	HNC—hydrogen isocyanide		Rotational	1(?), 3
	OCS—carbonyl sulphide		Rotational	3
	HCO^+—formyl ion		Rotational	1, 3
	HCO—formyl radical		Rotational	3
	CCH—ethynyl radical		Rotational	1(?), 2, 3
	N_2H^+—		Rotational	1(?), 3
4	NH_3—ammonia		Inversion	1, 3
	H_2CO—formaldehyde		K-doubling	1, 3, 4
			Rotational	1, 3
	HNCO—isocyanic acid		Rotational	3
	H_2CS—thioformaldehyde		K-doubling	3
			Rotational	3
	C_3N—cyanoethynyl radical		Rotational	2
5	HC_3N—cyanoacetylene		Rotational	1(?), 2, 3
	HCOOH—formic acid		K-doubling	3
	CH_2NH—methanimine		K-doubling	3
	H_2CCO—ketene		Rotational	3
	NH_2CN—cyanamide		Rotational	3
6	CH_3OH—methyl alcohol		K-doubling	3
			Rotational	3
	CH_3CN—methyl cyanide		Rotational	3
	NH_2CHO—formamide		K-doubling	3
			Rotational	3
7	CH_3C_2H—methyl acetylene		Rotational	3
	CH_3CHO—acetaldehyde		K-doubling	3
			Rotational	3
	NH_2CH_3—methylamine		Rotational	3
	CH_2CHCN—vinyl cyanide		K-doubling	3
	HC_5N—cyanodiacetylene		Rotational	3
8	$HCOOCH_3$—methyl formate		K-doubling	3
9	$(CH_3)_2O$—dimethyl ether		Rotational	3
	CH_3CH_2OH—ethyl alcohol		Rotational	3
	CH_3CH_2CN—ethyl cyanide		Rotational	3

*1, Dark dust cloud; 2, circumstellar envelopes; 3, H II region and/or galactic centre; 4, external galaxies.

to measure directly the $^{13}C/^{12}C$ ratio simply by measuring, for example, the abundance ratio $^{13}CO/^{12}CO$. Indeed it is strikingly apparent that for at least one important element, hydrogen, isotopic fractionation is very important in molecular clouds. The interstellar D/H ratio has been measured to be $\sim 10^{-5}$ by means of ultraviolet spectrometers on the Copernicus satellite. On the other hand, of the seven deuterated molecules that have been positively or possibly identified in molecular clouds (HD, DCN, DCO^+, DNC and deuterated water, ammonia, and methylamine), none have yielded abundances, in a ratio to the corresponding non-deuterated molecule, within an order of magnitude of 10^{-5}. HD, for example, also observed in the ultraviolet with the Copernicus satellite[18], is $\sim 10^6$ times less abundant than H_2, whereas the other six deuterated molecules, all seen by means of their radio spectra, seem typically to be only 10^2 times less abundant than their non-deuterated counterparts.

Despite these obvious fractionation effects, it seems that it may be possible to say something about the D/H ratio as a function of position in our Galaxy. Near the galactic centre DCN seems to be underabundant relative to its abundance in the vicinity of the Sun. This has been interpreted[19] to mean that deuterium itself is underabundant near the galactic centre where most of the observed gas has been processed through stars, in agreement with earlier notions from stellar evolution theory that nuclear burning in stars destroys D rather than produces it. If so, one is tempted to attribute the creation of the interstellar deuterium to the Big Bang explosion. In the simple case of a conventional general relativistic cosmology, a D/H ratio $\gtrsim 10^{-5}$ implies that our expanding Universe is open and will continue to expand forever.

Birth and death of stars

Historically, the stars have not been objects of affection for radio astronomers. Because of their poor angular resolution, radio telescopes are ill-suited to the study of minute objects such as stars, particularly since most stars do not emit much radio energy. Molecular radio astronomy is at a further disadvantage in that bright massive main-sequence stars that are burning hydrogen to helium in their interiors emit so much ultraviolet radiation that all nearby molecules are rapidly destroyed by photo-dissociation. During the past few years, however, it has become evident that the regions in our galaxy where interstellar molecules are observed are usually associated with either the birth or the death of a star or star cluster[20].

The most obvious signposts for star formation in our Galaxy are the hot, bright, blue O and B type stars that are 10^4–10^5 times as luminous as our sun and which are often surrounded by glowing clouds of ionised gas called H II regions. The Orion Nebula is the most famous H II region in the sky and is visible to the naked eye as the middle 'star' in the sword of Orion, the Hunter. Often associated with these O stars and H II regions are giant molecular clouds, which appear to be fragmented on at least three mass scales: 10^5, 10^3, and 10 times the mass of our Sun ($M_\odot$). O and B type stars are often found in associations composed of subgroups separated by 30–60 light years and strung out along the galactic plane[21]. It may be possible to identify strings of giant $10^5 M_\odot$ molecular clouds as the precursors of these OB sub-groups[22]. Each $10^5 M_\odot$ cloud in turn seems to fragment into $10^3 M_\odot$ pieces separated by distances $\sim$3 light years, and which may be precursors of galactic star clusters, such as the Trapezium, which is located at the centre of the Orion Nebula. Near the Orion Nebula, for example, there is a giant molecular cloud that appears to have fragmented into half a dozen or more pieces[23]. The fragmentation process may take millions of years.

The $10^3 M_\odot$ pieces fragment further into stars, many of which are seen as infrared continuum sources buried in the molecular clouds. Often associated with the infrared sources are bright OH (hydroxyl) and water masers, which come from regions comparable with or somewhat larger than the size of our Solar System. Perhaps, if we are both clever and lucky, we shall someday be able to use these masers as a tool for understanding the final stages in the formation of stars and associated planetary systems.

The superficial picture of star formation that we have pieced together above is still riddled with fundamental uncertainties. That is, we still have no clear understanding of motions in molecular clouds and, unlike the main-sequence phase in a star's life, motion is what star formation is all about. The relative importance of magnetic, rotational, gravitational and turbulent motions and forces in molecular clouds is still a subject of hot controversy. A few years ago it was suggested that the broad CO lines observed in molecular clouds could be best explained by free-fall collapse with velocity proportional to radius of large sections of the clouds[24,25]. But there are problems with such models[26,27], including, for example, excessive star formation rates. The recent observation[28] of self-reversed CO profiles in some molecular clouds suggest that, at least in these clouds, collapse is not very fast or v is not proportioned to r, or both. Although interpretation of these observations is still ambiguous[29], certain asymmetries present in the CO profiles suggest collapse velocities that are comparable with turbulent velocities. But other clouds have been seen with the opposite asymmetries in the CO profiles, thus suggestive of expansion rather than collapse.

If supersonic turbulence is present in molecular clouds, it will die out quickly unless it is regenerated or the clouds are very clumpy. The degree of clumpiness on size scales less than a few tenths of a light year is under investigation[30,31]. The part, if any, played by magnetic fields is still unclear. Some clever new ideas and/or observations are required if we are to improve our incomplete understanding of star formation.

The death of stars is a somewhat better understood process than their birth. This is because evolved stars, such as red giants, and dying stars, such as planetary nebulae white dwarfs and supernovae, have been studied optically for many years. Except for the white dwarfs, each of these types of star is known to be losing significant mass into interstellar space. Red giants and planetary nebulae are common objects and it is believed that most stars with masses greater than the mass of our Sun pass through both of these stages of evolution. Until very recently, however, it was not clear that anyone had actually seen stars that were evolving between the red giant phase and the planetary nebula phase.

An infrared survey from rockets[32] has been followed by ground based infrared[33–38] and radio molecular[39–41] observations of some of the very cool stars seen from the rockets. It now seems that the progenitors of planetary nebulae may be observable as very cool, extreme cases of red giant stars. The central stars in these progenitors are enshrouded in a cool circumstellar envelope composed primarily of molecules and dust particles. The presence of the cool dust is deduced from infrared 'excesses', often observed at wavelengths of 3–20 μm. The cool dust absorbs the visible and near infrared light emitted by the underlying star and by hotter dust and reradiates it at longer infrared wavelengths.

The gaseous molecular envelope is best studied by means of radio spectroscopy. Envelopes in red giant stars with more oxygen than carbon ('oxygen-rich') were first detected[42] eight years ago by means of OH maser radiation that they emit. Later, both water[43] and silcon monoxide[44] masers have been observed from many of these oxygen-rich stars. The apparent sizes of the OH and H_2O emission regions have been measured by trans-continental and inter-continental radio interferometers to be extremely small (of the order of the size of our Solar System). Because the strongest SiO masers are due to transitions between rotational levels in the first and second excited vibrational states which require energies of thousands of degrees for excitation, there is good reason to suspect that the SiO masers will come from even smaller regions than the OH and H_2O masers. (Because SiO is a stable molecule, it is not unreasonable that it might exist near or even on the stellar surface.)

Because we now know of three different molecules that produce high gain maser radiation in oxygen-rich stars and because the maser levels have different excitation energies, a wide range of conditions in the outer stellar atmospheres and circumstellar envelopes may be investigated with high spatial resolution. With such data in conjunction with the infrared observations we may hope to eventually gain a detailed understanding of mass loss mechanisms and of the physical conditions around evolved oxygen-rich stars[45]. In particular, it is already known that the OH and H_2O maser outputs follow intensity variations in the near infrared flux so that the masers are probably 'pumped' by near and/or far infrared radiation emitted by the star.

The other kind of evolved red giant stars, those with more carbon than oxygen in their outer layers, are rarer than the oxygen-rich stars discussed above. Recent radio observations[41] suggest that many of these carbon-rich stars may be losing mass at a rate comparable with the mass loss rates for planetary nebulae and significantly greater than the rate of mass loss from most oxygen-rich stars[46]. Unfortunately, largely by a quirk of fate, it is not possible to measure the carbon-to-oxygen ratio in planetary nebulae by optical techniques. An interesting problem for future investigation will therefore be to determine if most or only a relatively small fraction of planetaries pass through a carbon star stage.

Comparison of the lifetimes, compositions, distribution, kinematics and masses of planetary nebulae and carbon-rich and oxygen-rich infrared stars should lead to a clearer understanding of the physical mechanism responsible for the ejection of a planetary nebula envelope. It is also possible that the oxygen- and carbon-rich stars form two successive stages in an evolutionary sequence leading to planetary nebulae. It is already known that the distribution and kinematics of the oxygen-rich, variable stars are similar to those of the planetary nebulae. It is therefore conceivable that red giant stars first shed their oxygen-rich outer envelopes at a relatively leisurely pace and thereby reveal a carbon-rich interior region which is then shed more rapidly to form a planetary nebula. Whether this scenario is representative for most or only a few stars awaits further observational tests.

Galactic and extragalactic structure

Because the Milky Way contains an appreciable amount of dust that scatters and absorbs light waves from distant stars, it was not until the advent of spectral line radio astronomy in the 1950s that a complete map of the Milky Way could be constructed. The maps were of the intensity of 21-cm wavelength radio waves emitted by neutral atomic hydrogen located throughout the Galaxy. In conjunction with a simple model for the revolution of mass about the galactic centre, it was possible to plot the distribution of hydrogen atoms in the Galaxy. It was assumed that this map would also represent the distribution of other young constituents in the Galaxy (by 'young' we mean objects such as bright massive stars and their remnants (for example pulsars), which are believed to have formed out of the gas within the past few times 10×10^6 yr). Some astronomers were therefore surprised when the first large scale maps[47–49] of the very abundant interstellar carbon monoxide molecules showed a distribution that is very different from the distribution of hydrogen atoms. The molecular maps indicate that the CO clouds are concentrated near the centre of our pancake-shaped Galaxy, as well as in a ring lying between 4 and 8 kiloparsecs (kpc) from the centre. Very few molecular clouds are located at or beyond the circle of radius 10 kpc that our Solar System traverses as it orbits around the galactic centre. The atomic maps, on the other hand, indicate a fairly uniform density of hydrogen between 4 and 14 kpc from the galactic centre. Therefore, the bulk of the atomic gas lies outside of the solar circle. Since the CO is believed to trace out the distribution of the (unobservable) H_2 molecule, the inescapable conclusion is that atomic (H) and molecular (H_2) clouds in our Galaxy have very different distributions.

The explanation for this difference is not yet certain, but it probably has something to do with the large scale spiral structure of the Galaxy. That is, the 'typical' low density atomic hydrogen cloud, or group of clouds, is not sufficiently massive to become gravitationally unstable and collapse to form denser clouds where H_2 molecules can be formed and protected. Help is needed and such help is probably provided by a spiral density wave that produces shock fronts and compresses the atomic clouds in the inner, but not the outer parts of the Galaxy[47]. Some of the compressed clouds or groups of them become gravitationally unstable and can collapse. (The collapse process might also involve the galactic magnetic fields.) Because current star formation is believed[20] to be more closely tied to molecular, rather than atomic clouds, it is not surprising that the best available pictures of the distribution of massive young stars[50] (indirectly visible at great distances from the sun by the radio waves emitted in the extensive hot plasmas that surround them) and supernova remnants[51] agree much better with the CO maps than with the 21-cm hydrogen maps.

Maps[52] of the galactic γ-ray (energies $\gtrsim 100$ MeV) distribution also show a remarkable resemblance[53] to the CO maps. Because the most likely γ-ray production mechanism is interaction of cosmic ray nucleons with interstellar gas nuclei, the γ-ray maps trace out a product of the densities of these two galactic constituents. The data suggest[53,54] that the cosmic ray intensities are somewhat enhanced in the general vicinity of the molecular clouds and the supernova remnants and that cosmic rays, at least those of energies 1–10 GeV, originate in the Galaxy (as opposed to outside it).

Because of the widespread distribution of CO in the Milky Way, it has been expected that the CO rotational spectrum would also be observed from other galaxies. Recently, however, radio astronomers finally detected extragalactic CO near the centre of one active irregular, four active spiral galaxies[55], and in the Large Magellanic Cloud[56], a very nearby irregular galaxy, easily visible with the naked eye from the Southern hemisphere. By 'active' we mean that the galactic central regions emit strong infrared and radio frequency continuum radiation and optical emission lines, as well as showing evidence for non-circular motions and mass ejection. These are also characteristics of the centre of the Milky Way, albeit on a smaller scale. Because the single largest concentration of molecular clouds in our Galaxy is located near its centre, it is not surprising that the nuclei also contain the most impressive concentrations of molecules in the active galaxies. In all of these galaxies, including our own, the molecular clouds and energetic phenomena are probably associated with star formation and death. Gravitational forces tend to enhance the nuclear densities, which result in massive molecular clouds and relatively rapid rates of formation of stars which excite the interior regions and might lead to violent (explosive) events such as supernovae. With somewhat more sensitive receivers, millimetre wavelength interferometers, and larger telescopes it should be possible to greatly expand our knowledge of the distribution of extragalactic molecules. Higher angular resolution, for example, would enable radio astronomers to map the (spiral) structure of other galaxies and to compare the molecular distribution with the distribution of dust, atomic gas, emission nebulae, and synchrotron radiation. Better sensitivity will allow study of the less abundant isotopes of CO and other molecules which in turn will help us to understand the chemical history of these galaxies.

Summary

The study of interstellar molecules during the past decade has been remarkably productive. Future prospects look equally bright. New large telescopes and interferometers sensitive to millimetre wavelength radiation will permit substantially more detailed study of molecules in regions of stellar birth and death in the Milky Way and external galaxies. New instrumental techniques have already pushed the short wavelength frontier to sub-millimetre wavelengths (the observation[57] of the $J=3\rightarrow2$ transition of CO on the 200-inch Hale telescope). Portions of the millimetre and sub-millimetre spectrum, not accessible from the ground because of atmospheric absorption, will be observable from aircraft, balloons and satellites. Initial steps in this direction have been taken[58] with a 183 GHz radiometer flown on the G. P. Kuiper Airborne Observatory.

Perhaps the most fertile, as yet largely unexplored, area of the spectrum is the infrared, where high resolution spectroscopy is still in its infancy. H_2 rotation–vibration transitions near 2-μm wavelengths have recently been detected[59] for the first time in interstellar space towards the Orion Nebula and a planetary nebula.

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articles

β-Sheet topology and the relatedness of proteins

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The topological connectivities of β-pleated sheets in the known protein structures are systematically surveyed for regularly occurring features. The many empirical restrictions found and the distribution of actually occurring topologies among the hypothetically possible ones suggest that striking topological similarities between different proteins can readily happen by chance.

β-PLEATED sheets are major features in about 80% of the globular proteins of known three-dimensional structure, and β sheets inherently require the close interaction of regions separated in the amino acid sequence. An understanding of β sheets is therefore central to an understanding of the organisation and folding of proteins, and will probably be essential in any successful method of predicting three-dimensional structure from amino acid sequence. A number of particular regularities have been noted in β structure[1–4], but there has been no systematic search for such features.

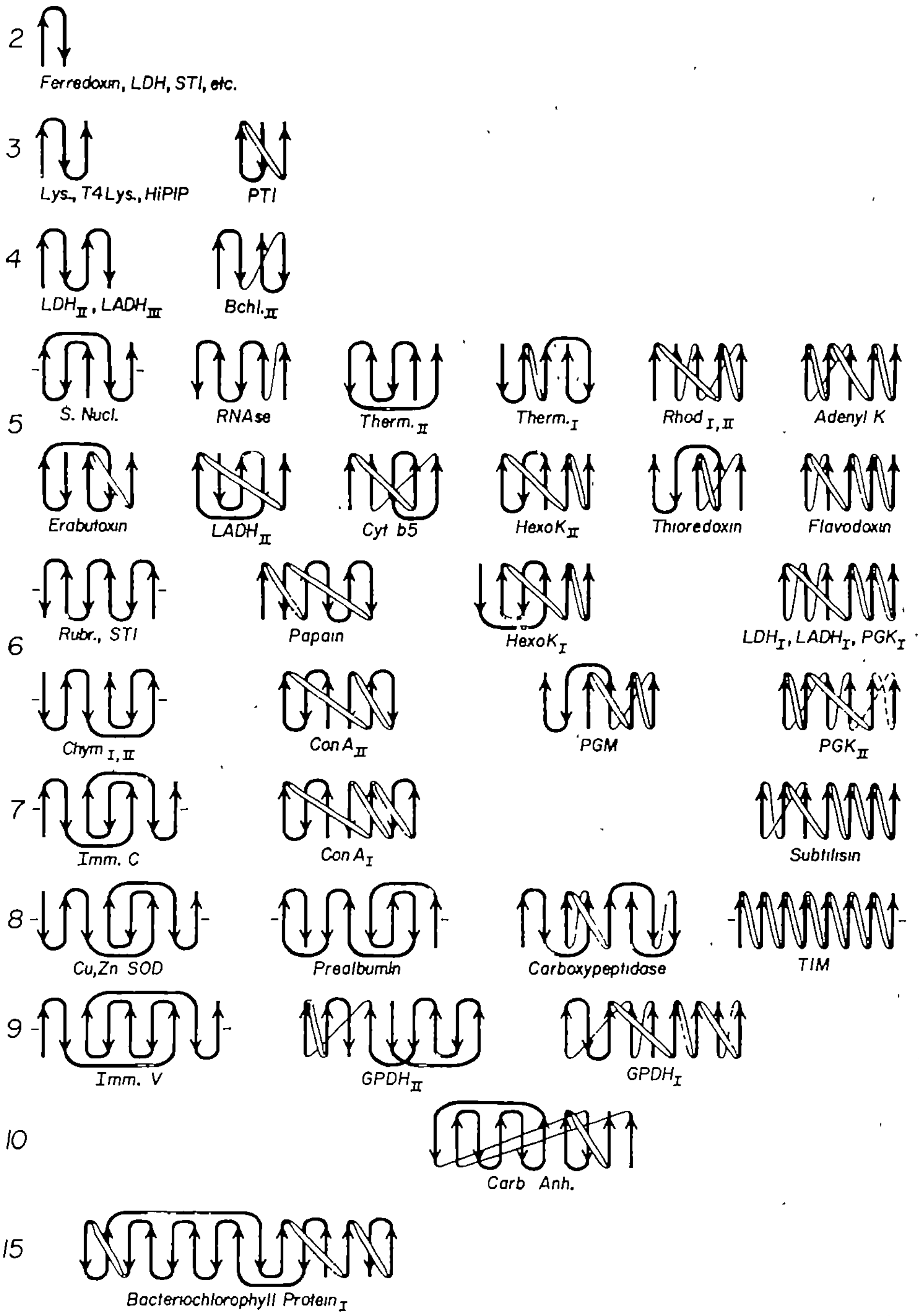


Fig. 1 Schematic diagrams for all the topologically-distinct β-pleated sheets found in proteins of known three-dimensional structure. Arrows indicate the strands of the β sheet, considered to be in the plane of the paper; crossover connections shown wide are above the sheet and thin ones are below it. Only topological connectivity is represented, with no attempt to indicate the length or conformation of the connections. The β sheets are sorted from top to bottom by number of strands, and from left to right according to their percentage of antiparallel as against parallel β structure. In cases of proteins with more than one β sheet, roman numeral subscripts are added. Those β sheets which can be considered to form cylindrical barrels are flanked by hyphens and are shown as viewed from the outside. Abbreviations and literature references for the 33 proteins included (in decreasing order of β-sheet size) are as follows: bacteriochlorophyll protein (Bchl)[8], carbonic anhydrase C (Carb. anh.)[9], glyceraldehyde phosphate dehydrogenase (GPDH)[10], immunoglobulin variable (Imm. V) and immunoglobulin constant (Imm. C) domains[11], Cu,Zn superoxide dismutase (Cu,Zn SOD)[12], prealbumin[13], carboxypeptidase A[14], triose phosphate isomerase (TIM)[15], concanavalin A (Con A)[16], subtilisin[17], chymotrypsin (Chym.)[18], phosphoglycerate mutase (PGM)[19], phosphoglycerate kinase (PGK)[20], rubredoxin (Rubr.)[21], soybean trypsin inhibitor (STI)[22], papain[23], hexokinase (HexoK)[24], lactate dehydrogenase (LDH)[25], liver alcohol dehydrogenase (LADH)[26], erabutoxin[27], cytochrome b5 (Cyt. b5)[28], thioredoxin[29], flavodoxin[30], staphylococcal nuclease (S. nucl.)[31], pancreatic ribonuclease (RNAse)[32], thermolysin (Therm.)[33], rhodanese or thiosulphate sulphurtransferase (Rhod.)[34], adenyl kinase (Adenyl K)[35], egg white lysozyme (Lys.)[36], T4 phage lysozyme (T4 Lys.)[37], high-potential iron protein (HiPIP)[38], pancreatic trypsin inhibitor (PTI)[39]; ferredoxin[40].

Similarity of the topological connectivity of β-sheet strands has been central to consideration of the possible evolutionary relationship between distantly similar proteins[1-7], but it has been very difficult to evaluate the significance of such topological similarities. Here I introduce both a graphical and a notational convention for representing the topological connectivity of β sheets, and use this highly simplified representation to systematically survey the known β sheets for regular patterns. Possible implications for the folding and for the evolutionary relatedness of proteins are pointed out.

The possible backbone connections between β strands fall into two general categories: 'hairpin' connections in which the backbone chain re-enters the same end of the β sheet it left, and 'crossover' connections in which the chain loops around to re-enter the sheet on the opposite end (illustrated in Fig. 1 of ref. 2). Starting from the N-terminal strand, each connection is named by how many strands it moves over in the sheet and in which direction, with an 'x' added for crossover connections. Thus a '$+1$' is a hairpin and a '$+1x$' is a crossover connection between nearest-neighbour strands; a '$+2$' is a hairpin and a '$+2x$' is a crossover connection that skips past one intervening strand in the sheet, and so on. As an example, the five-stranded β sheet of thioredoxin (see Fig. 1) can be described as either $-2x, +1x, -2, -1$ or as $+2x, -1x, +2, +1$ (absolute value of the signs is not meaningful, since the diagram could be turned upside down).

Figure 1 shows simplified topological diagrams for the 37 topologically distinct β-sheet structures found in proteins of known three-dimensional structure. Assignment of these β-sheet topologies was made from examination of stereo drawings, hydrogen-bonding diagrams, and published descriptions of the structures. In some cases a decision must be made about inclusion or omission of a particular extended strand: very short strands which turn abruptly away after making one hydrogen bond were generally omitted if they were at an edge of the sheet, while long extended strands which stay close to a neighbouring strand are generally included even if they are not known to form hydrogen bonds (for example, a fifth parallel strand in rhodanese). Also, each continuous stretch of extended chain is treated as a single β strand; this is the usual convention but differs, for example, from the usage in ref. 4. All of these β sheets have the usual twist[1], although that is not shown in these schematic diagrams. All consideration of length and conformation in the connecting backbone chains is omitted, in order to concentrate on the purely topological features. Figure 1 thus summarises the empirical data base from which all of the following conclusions are drawn.

Handedness of crossover connections

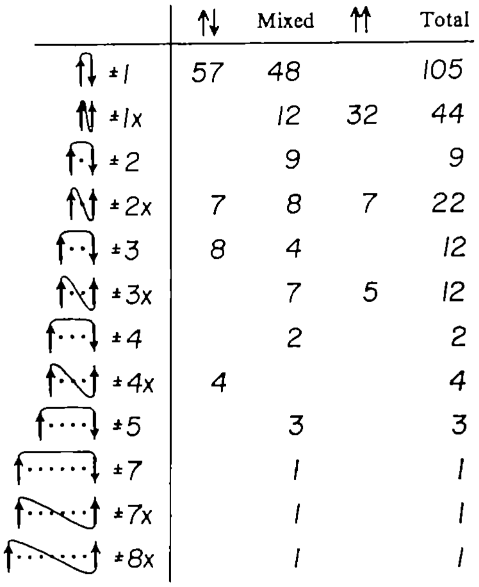
All crossover connections are right-handed (except for one, in subtilisin). That is, in Fig. 1, crossover connections above the β sheet all slant down to the right, and crossover connections below the sheet all slant down to the left. This handedness phenomenon is discussed in detail elsewhere[2].

Parallel β structure only in large, protected sheets

No parallel β structure occurs in sheets of less than five total strands. This suggests that considerable cooperativity is necessary to stabilise parallel β structure, whereas antiparallel β structure often occurs as a twisted ribbon of just two strands.

Parallel β sheets and the parallel portions of mixed sheets are always thoroughly buried, with other main chain (often α helices) covering them on both sides. Antiparallel sheets, on the other hand, typically have one side exposed to solvent with only the rather sparse protection of the side chains. This pattern also suggests a lower stability for parallel β structure, since apparently it cannot tolerate solvent access to its hydrogen bonds. The necessity for considerable protein structure on both sides of a parallel β sheet (as well as the need for loops to make the crossover connections) means that parallel sheets occur only in relatively large protein domains. For domains built around a parallel β sheet the average size is 179 residues with a minimum of about 135, whereas for domains built around a large antiparallel sheet the average size is 128 residues and the minimum is 54.

For right-handed crossover connections to shield both sides of a non-cylindrical parallel β sheet, there must be at least one change of direction (change of sign in the written convention). Apparently these direction changes are somewhat unfavourable for parallel sheets, since none of the nine pure parallel β sheets change direction more than once, although for antiparallel and mixed sheets of similiar sizes 11 out of 20 examples change direction two or three times.



	↑↓	Mixed	↑↑	Total
±1	57	48		105
±1x		12	32	44
±2		9		9
±2x	7	8	7	22
±3	8	4		12
±3x		7	5	12
±4		2		2
±4x	4			4
±5		3		3
±7		1		1
±7x		1		1
±8x		1		1

Fig. 2 Occurrence frequencies of each connection type in the known β sheets. The number of occurrences in pure antiparallel, in mixed, and in pure parallel β sheets are tabulated separately, and are totalled for all β sheets of 5 or more strands.

Connection type frequencies

Figure 2 gives the number of occurrences of each connection type. Of the connections that occur, 70% are either ±1 or ±1x. This fact alone is sufficient to make some β-sheet topologies much more favourable than others, as has previously been pointed out[6].

Asymmetries of chain direction

For almost all local (2 or 3 strand) topological features, occurrence frequencies seem to be indifferent to backbone chain direction. One exception is that the N-terminal strand is somewhat more likely than the C-terminal strand to be in the middle of the β sheet: 15 N-terminal strands are at an edge, 4 are in by one strand, and 14 are in by more than one strand; while 22 C-terminal strands are at an edge, 7 are in by one strand, and only 4 are in by more than one strand. This supports the view that there is a tendency for the earliest folding nucleation sites to be near the beginning of the chain.

In general, reversal of chain direction produces a topologically distinct structure (except in occasional symmetrical cases, such as chymotrypsin and the immunoglobulin domains); however, since occurrence frequencies of all the local patterns except the buried N termini are indifferent to chain reversal, the following discussion assumes that a given topology and its reversed version have the same probability of occurrence.

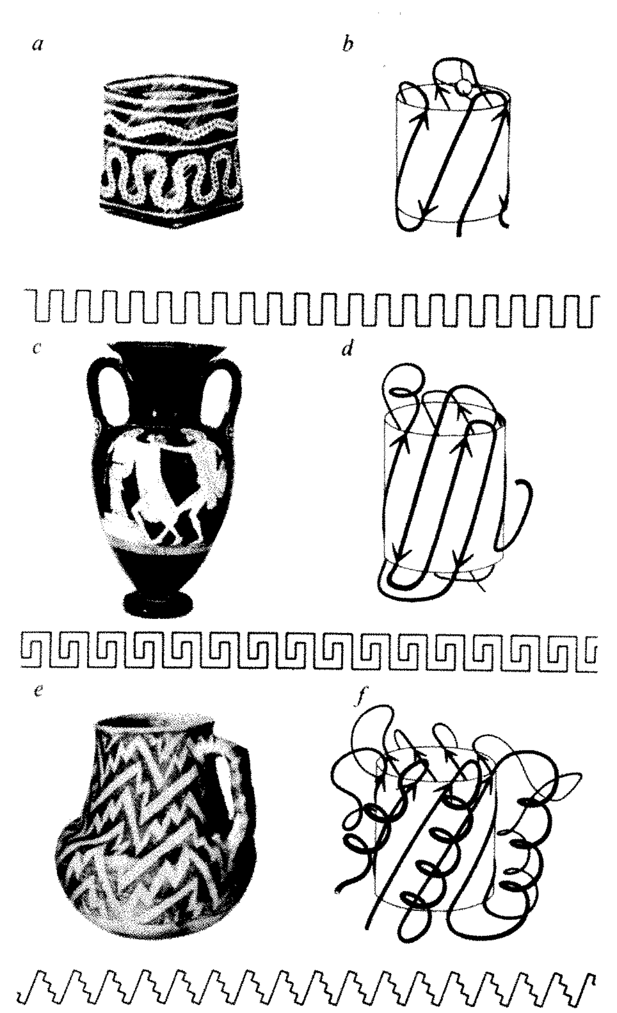


Fig. 3 Comparison of geometric motifs common on Greek and American Indian weaving and pottery with the backbone folding patterns found for cylindrical β-sheet structures in globular proteins. *a*, Indian polychrome cane basket, Louisiana. *b*, Polypeptide backbone of rubredoxin. *c*, Red-figured Greek amphora showing Cassandra and Ajax (about 450 BC). *d*, Polypeptide backbone of prealbumin. *e*, Early Anasazi Indian redware pitcher, New Mexico. *f*, Polypeptide backbone of triose phosphate isomerase. Photographs (*a*) and (*e*) courtesy of the Museum of the American Indian, Heye Foundation; photograph (*c*) courtesy of the Metropolitan Museum of Art, Fletcher Fund, 1956.

Simplicity of commonest topological patterns

Among locally interconnected 4-strand regions, the only topologies that occur more than 3 times are: $+1,+1,+1$ 16 times; $+3,-1,-1$ 11 times; $+1x,+1x,+1x$ 6 times; and $+1x,+2x,-1x$ 5 times. These frequencies are about what would be expected from the occurrence frequencies of the individual connection types making them up (see Fig. 2), except that $+3,-1,-1$ occurs more often than expected. Perhaps one reason why that pattern is especially favoured is that besides folding up one strand at a time it can also fold by forming a long 2-stranded ribbon which then bends in the middle.

The distinctive topological patterns which are seen in the β-barrel structures are the simple up-and-down 'meander' pattern of $+1,+1,+1$ (as in rubredoxin and soybean trypsin inhibitor); the 'Greek key' pattern of $+3,-1,-1,+3$ or just $+3,-1,-1$ (as in staphylococcal nuclease, chymotrypsin, immunoglobulins, superoxide dismutase and prealbumin); and the strict alternation of parallel β with helix in the 'lightning' pattern of $+1x,+1x,+1x$ (as in triose phosphate isomerase). As shown in Fig. 3, each of these patterns corresponds to a common geometric motif used to decorate weaving and pottery. Besides providing a useful mnemonic for the β-barrel structures, this comparison emphasises the fact that, like good pottery decoration, the topological patterns found in β structure are not haphazard collections of lines but are subject to constraints of simplicity and good design.

For parallel β barrels, since crossover connections must be right-handed and cannot go down the centre of the barrel, a rigorous repetition of $+1x,+1x,+1x$ in the lightning pattern is the only likely topology. Therefore it is to be expected that any parallel β barrels found in the future will very closely resemble triose phosphate isomerase, whether they are related to it or not.

The Greek key topology of $+3,-1,-1,+3$ is symmetrical to chain reversal, but when it occurs on a barrel it has two mirror-image forms, depending on whether the swirl is clockwise or counterclockwise as viewed from the outside of the barrel (see Fig. 3 insert and cover). For all five proteins with Greek key β barrels, the swirl is counterclockwise. This could simply be a strong coincidence; however, it seems likely that the counterclockwise swirl either folds more easily or is a stable structure (the two forms are not exact mirror images, because the β strands always have a right-handed twist and the amino acids are asymmetrical).

Preference for pure over mixed sheets

The ratio of the number of pure parallel or pure antiparallel β sheets to the number of mixed sheets is very much greater than would be expected from a random distribution. In our sample there are 124 antiparallel and 73 parallel strand pairs in the sheets of at least 5 strands. If the parallel and antiparallel probabilities are considered approximately equal and if they were independent from one pair to the next across the β sheet, then there should be approximately 2 pure sheets (one parallel and one antiparallel) for every 14 mixed sheets in 5-stranded cases, and 2 pure for every 30 mixed in 6-stranded cases. Since Fig. 1 contains 12 5-stranded and 8 6-stranded sheets, the expectation would be 2 pure and 18 mixed sheets if the probabilities were independent; in fact there are 12 pure and only 8 mixed sheets out of the 20.

It is easy to understand why the parallel and antiparallel probabilities are not independent from one strand pair to the next: since the ideal conformations for the two types of β structure are slightly different, a strand which has antiparallel hydrogen-bonding on one side is already set up for antiparallel hydrogen bonds on the other side. Indeed, it turns out that 138 strands have the same type of bonding on both sides, while only 33 have opposite bonding types on the two sides.

One interesting result of this strong preference for pure rather than mixed β sheets is that although the current set of known protein structures is beginning to provide a statistically meaningful sample of the pure β sheets (18 with >4 strands), the 14 mixed sheets are actually 15 times sparser sampling out of the possible cases, and therefore very little can yet be said about them.

Absence of knots

There are no knotted topologies within the known β sheets—that is, no backbone β-sheet configurations which, if considered as a rope grasped at N and C termini and pulled out straight, would end up with a knot in the rope. (However, the backbone of carbonic anhydrase C contains a knot involving the first 30 residues (not part of the β structure) and the last 20 residues (which do include a β strand). (Presumably a knot is not impossible if the piece to be tucked through the loop is very close to a chain end.) It has often been pointed out (for example ref. 41) that it would be exceedingly difficult for a protein to fold up into a knotted configuration. This is not a trivial restriction, since a relatively large number of the hypothetically possible topologies are knotted, especially for parallel sheets. The simplest such case is $+2x, -3x, +2x$, —the one knotted topology out of 12 (if left-handed crossover connections are excluded) for a 4-stranded pure parallel sheet. Of the possible topologies, 25% are knotted for a 5-stranded pure parallel sheet, and 45% of the topologies are knotted for a 6-stranded pure parallel sheet.

Adequacy of a simple nomenclature

One set of restrictive conditions can be summarised by saying that it is almost always possible to describe a real β-sheet topology by a list of connection types. Many of theoretically possible arrangements which would violate this restriction simply do not occur: for instance, two long β strands hydrogen bonded to each other as nearest neighbours at one end but hydrogen-bonded to an intervening β strand at the other end.

Ambiguous chain crossings are also extremely rare in real structures (that is, connections which would be topologically different depending on which of two connection chains was on top). There is just one such case involving hairpin connections in $\mathrm{GPDH_{II}}$ (see Fig. 1), and just one such case involving crossover connections, in subtilisin (see Fig. 1). Often one version of a hypothetical ambiguous pair is knotted; the two versions are not counted as separate topologies in this paper.

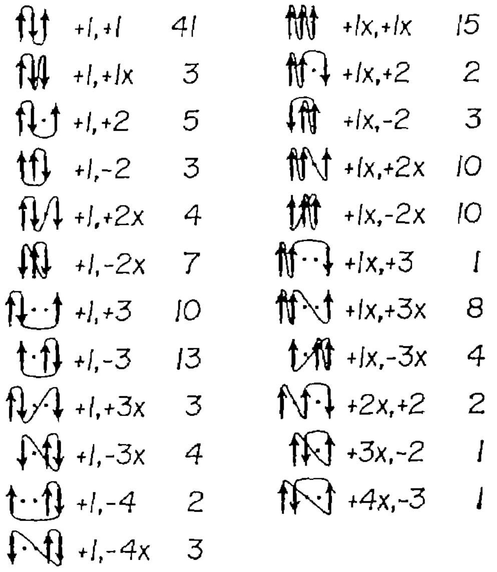


Fig. 4 Occurrence frequencies for consecutive (in the amino acid sequence) pairs of connection types in β sheets of five or more strands. A connection pair and its reversal (for instance, $+1x$, $-2x$, and $-2x$, $+1x$) are tabulated together. Connection pairs that span more than five strands are omitted.

Preference for topologies with many folding pathways

It is possible to assume a hypothetical set of rules for allowable steps of protein folding for β sheets, more or less in agreement with ideas which have often been suggested before[3,42–45]. Assuming that the α helices and β strands are already present at least statistically, let us say that each succeeding step in any possible folding pathway for a β sheet must consist of either (1) forming a ± 1 or $\pm 1x$ connection between two β strands adjacent in sequence, or (2) taking either a β strand or a prefolded unit and laying it down next to a prefolded part of the sheet with which it is also contiguous in sequence. Many hypothetical topologies which do not occur are unfoldable by this definition, including all the knotted ones. But the occurring β sheet topologies typically have a large number of such possible folding pathways (the notable exceptions are subtilisin, with no such pathways because the left-handed $+1x$ lies outside of the $-2x$, and cytochrome b5, with only one). Especially for cases with 10 or 20 potential folding pathways it seems unlikely that one is so much more favourable that it effectively dominates all the others. Instead, it may be that one reason that these structures are favourable is because they can fold correctly by many alternative pathways and therefore tend to fold rapidly and reliably. The idea that some broad features of protein structure are determined by essentially kinetic factors during folding (such as the dominance of near-neighbour strand interactions) is not incompatible with the requirement that the stable native conformation of a protein should be in the global free energy minimum. During its evolution, once a protein finds a reasonably stable, kinetically accessible, minimum energy conformation, it is then subject to natural selection for stability. Selection will dig that particular local energy minimum as deep as possible by adjusting the amino acid sequence to approach optimal fit for the native conformation, and at the same time probably will raise all the other local energy minima whose stability is not being selected for. The result is very likely to be a genuine global energy minimum, in spite of the influence of kinetic requirements.

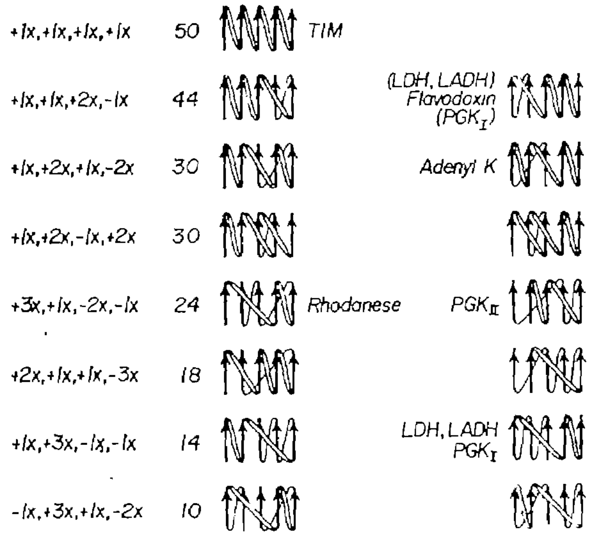


Fig. 5 The most probable topologies for 5-stranded pure parallel β sheets. The first column lists the sequence of connection types. Numbers in the second column are normalised pseudo-probabilities obtained by multiplying occurrence frequencies of the three pairs of connection pairs as described in the text. The diagrams in the last column are obtained by reversal of the backbone chain direction. Next to each diagram are listed all proteins which contain that topology as a contiguous unit (both in sequence and within the β sheet), the protein is in parentheses if a single strand at one end of either the sequence or the sheet was ignored to make the 5 strands contiguous See Fig. 1 legend for abbreviations.

Probability ordering for β topologies and implications for evolutionary relatedness

Figure 4 gives the occurrence frequencies for various consecutive pairs of connection types. These pair frequencies can be used to generate pseudoprobabilities which give a relative ordering among possible β-sheet topologies.

As an example, consider the pure parallel and the pure antiparallel cases, for 5-stranded β sheets. With left-handed crossover connections eliminated (see above), parallel and antiparallel cases each have $n!\ /2 = 60$ possible topologies, consisting of 32 basic patterns and 28 others obtained by reversal of chain direction (4 of the topologies are symmetrical to chain reversal). Each 5-stranded topology contains 4 connections and 3 connection pairs. Its pseudoprobability is defined as the number obtained by multiplying together the occurrence frequencies of its three connection pairs (from Fig. 4, using a frequency of 1 if the pair did not occur in the data base), dividing by 2 for the symmetrical cases, and then dividing by a factor of 34 which normalises the +1,+1,+1,+1 case to 1,000.

Figures 5 and 6 show all pure parallel and all pure antiparallel 5-stranded topologies that have pseudoprobabilities greater than 8. The data sample is large enough (155 connection pairs) so that the pseudoprobabilities respond primarily to the overall connection pair frequencies and are only slightly affected by whether or not the exact 5-stranded topology in question occurs in the data base. The pseudoprobabilities could be further improved by inclusion of factors disfavouring knots, ambiguous chain crossings and buried C termini (see above). Also, for purposes that did not need to consider topologies embedded within larger sheets, it would be useful to include the probability of each connection type being the first or the last. But, the single simple criterion used in Figs 5 and 6 succeeds in including all occurring 5-stranded topologies on a rather short list, and the occurring topologies are a fairly dense sample of these 'most favourable' cases.

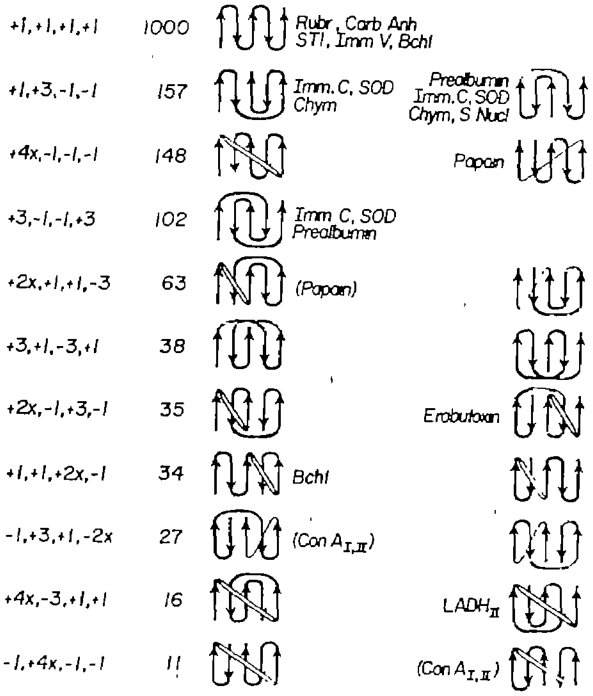


Fig. 6 The most probable topologies for 5-stranded pure antiparallel β sheets. All pure five-stranded topologies which occur as contiguous units in one of the known β sheets are included on the lists of Figs 5 and 6, except for the case of subtilisin, which would match the adenyl kinase entry if it did not have a left-handed crossover connection.

From the overall distribution of examples seen in Figs 5 and 6, it can be concluded that many of the protein pairs which have identical or closely similar β-sheet topologies merely represent coincidentally close choices among the rather few highly probable topologies. There must be examples among such proteins which resemble each other because of a true evolutionary relationship; however, topological similarity of their β structures provides only a very weak argument for such evolutionary relationship, although it is probably a necessary condition. It is suggestive that fully 75% of the pure β sheets are topologically similar to some other one, whereas among the mixed β sheets, for which the current sampling is 15 times sparser (see above), the only topologically similar pair are the two halves of hexokinase. At one extreme, evolutionary relationships almost certainly underlie the similarity of the nucleotide-binding domains of LDH and LADH, and the internal duplications of structure in rhodanese and in the immunoglobulins. At the other extreme, there are almost certainly not evolutionary relationships underlying the topological similarity of the two interdigitated β sheets in con A or underlying the topological similarity of proteins whose overall structural organisation is as different as, for instance, staphylococcal nuclease and Cu,Zn superoxide dismutase. The many cases intermediate between these extremes must each be decided on the basis of other evidence besides topology, but it seems likely from overall statistics that the bulk of them are coincidences produced by the fact that the number of highly favourable chain folding patterns is actually quite limited.

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Nerve growth factor-induced increase in electrical excitability and acetylcholine sensitivity of a rat pheochromocytoma cell line

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A cloned line of rat pheochromocytoma cells (PC12) which responds to nerve growth factor (NGF) by cessation of division and extension of neurone-like processes, is shown to develop electrical excitability and greatly increased acetylcholine sensitivity in the presence of NGF. This implies that NGF influences the physiological differentiation of certain neural crest derivatives.

LITTLE is known about the mechanisms which regulate the differentiation of neuroblasts into mature neurones. Cell cultures of normal or neoplastic origin have become increasingly useful model systems with which to study this otherwise difficult problem[1]. Recently a clonal cell line of rat pheochromocytoma cells (named PC12) was established which undergoes neurone-like morphologic differentiation in the presence of nerve growth factor (NGF) (ref. 2). NGF is a well characterised protein which stimulates neurite outgrowth from sympathetic neurones and which is thought to have an important role in their normal growth and development[3]. After several days in the presence of 10^{-10} M NGF, PC12 cells cease to divide and extend long neurite-like processes. In common with sympathetic neurones, PC12 cells (both NGF-treated and -untreated) also synthesise, store, take up, and release catecholamines[2,4,5]. Such findings suggest that in the presence of NGF, PC12 pheochromocytoma cells differentiate into sympathetic-like neurones.

Among the most characteristic properties of differentiated nerve cells are their electrical excitability and sensitivity to neurotransmitters. In this study, we have therefore chosen to establish whether PC12 cells would generate all-or-nothing actions potentials, if they would respond to acetylcholine (ACh) and if these properties were affected by NGF.

PC12 cells were grown in 35-mm collagen-coated Falcon plastic tissue culture dishes as previously described[2]. 2.5 S NGF (ref. 6) at 50 ng ml^{-1} was added to some cultures. The cells grown without NGF remained relatively spherical (between 6–14 µm in diameter) and did not extend processes. Cells grown in the presence of NGF for longer than 1–2 weeks were, on the average, slightly larger (10–15 µm in diameter) and most cells had one or more long processes. Physiological experiments were performed at 35–37 °C in medium containing 6 mM glucose, 145 mM Na, 3 mM K, 1.8 mM Ca, 1.0 mM Mg, 153 mM Cl buffered by 2.5 mM phosphate or 2.5 mM Tris to *p*H 7.4. The cultures were placed on the stage of an inverted phase contrast microscope and the cells were impaled under direct vision with microelectrodes containing 3.5 M potassium acetate (R = 40–100 MΩ) (ref. 7). The cells were stimulated with intracellular depolarising pulses of 50-ms duration passed through the recording electrode using a modified Wheatstone bridge. In many cases, in order to increase activation of spike generating mechanisms, the cells were hyperpolarised by passing negative background current through the microelectrode before depolarising pulses were applied.

Since PC12 cells are small and therefore subject to injury with the microelectrode, several criteria were used to control for this parameter when comparing spike generative capacity and ACh sensitivity of cells cultured in different conditions. First, recordings were included in our results only if the cell maintained a resting potential of −20 mV or greater. This excluded severely damaged cells. Second, the mean resting potential of each group of cells was determined. While such measurements do not reflect the absolute resting potentials of the unimpaled cells, they do provide a means of comparing the extent of cell damage. Finally, in many cases, comparisons were made with cultures studied on the same day and with the same set of microelectrodes.

Electrical excitability

In more than one hundred impaled PC12 cells with resting potentials greater than −20 mV, from eight different cultures, no cells showed action potentials (Fig. 1*a* and Table 1). This included 58 cells with membrane potentials (V_m) greater than −30 mV and 19 cells with V_m greater than −40 mV. In all cases, the cells were hyperpolarised to between −70 and −100 mV in order to maximally activate any possible spike-generating mechanism present. In two cells, after background hyperpolarisation, depolarising pulses produced small regenerative responses (<10 mV) which could just be distinguished from the delayed rectification which was often present.

In some experiments, more calcium (5.4–10.8 mM) was added to the medium to act as a membrane stabiliser and to enhance any Ca-dependent regenerative responses. Action potentials were not detected in 18 impaled cells (including 9 cells with $V_m \geqslant -30$ mV and 5 with $V_m \geqslant -40$ mV) in these conditions.

As noted above, delayed rectification responses were frequently observed (Fig. 1*a*) indicating that the mechanism controlling the delayed increase in K conductance of action potentials is present in these cells. Previous experiments have suggested that PC12 cells not treated with NGF also possess at

Table 1 Electrical excitability of PC12 cells grown in different conditions

Growth condition	Cell division	Process	Action potentials*	Mean V_m
NGF-untreated	+	−	0/111	31±1
NGF-treated (>2 weeks)	−	++	29/42	33±2
Ara-C (0.25–3 µM)	−	−	11/91	38±1
BUdR (3 µM)	−	−	0/15	35±2
Ara-C+NGF (>2 weeks)	−	++	49/65	44±1

*Numbers indicate cells with action potentials per total cells sampled with V_m equal to or larger than −20 mV.

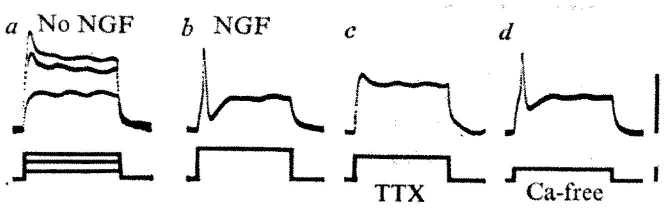

Fig. 1 Electrophysiological responses of PC12 cells. *a*, PC12 cell not treated with NGF. Three superimposed intracellular depolarising pulses (voltage trace above; applied current below) do not evoke a regenerative response. Delayed rectification is seen in the two larger responses ($V_m \simeq -62$ mV*). *b–d*, PC12 cells treated with NGF for more than 2 weeks. *b*, Depolarising pulse evokes action potential in PC12 cell bathed in control medium ($V_m \simeq -58$ mV*). *c*, Depolarising pulse fails to evoke regenerative response in another PC12 cell in presence of 3×10^{-7} M TTX. ($V_m \simeq -54$ mV*). *d*, PC12 cell bathed in Ca-free medium; action potential similar to that seen in *b*. ($V_m \simeq -70$ mV*). Calibration is 50 mV, 1 nA and depolarising pulse is 50 ms. (*, Indicates approximate V_m with background hyperpolarisation).

least some Na action potential ionophores, since veratridine, an alkaloid which specifically activates such sites[8], stimulates release of endogenous catecholamine stores[4]. The inexcitability of untreated PC12 cells could derive from too low a density of such ionophores or from the absence of another component of the action potential generating mechanism or for some other reason.

In PC12 cultures maintained in the presence of NGF for more than 2 weeks, most impaled cells demonstrated all-or-nothing action potentials when depolarised by passing current through the microelectrode (Fig. 1*b* and Table 1). Most often the cells required some background hyperpolarisation to between −50 and −100 mV before the depolarising pulse could elicit an action potential. Resting membrane potentials varied between −20 and −70 mV. In two experiments combined, 22 of 25 consecutively impaled cells showed active responses. In other experiments, presumably because of injury, active responses were found in only 50–80% of penetrated cells. The action potential morphology exhibited by these cells was varied but no cells showed prolonged plateaus, as may be seen in some murine neuroblastoma cells[9]. It could not be determined how much of this variability was attributable to differences in the population or was a function of impalement injury.

Most of the cells in the NGF-treated cultures had long (>100 μm) processes and thus it was possible that action potential generation was directly related to neurite outgrowth. In order to test this, those few cells in NGF-treated cultures which lacked visible processes were impaled. Such cells were still capable of generating action potentials. In another experiment, cells were grown in NGF for 3 weeks and then detached from the culture dish and subcultured into new media with NGF. As a result of this treatment, the cells lost their processes, mainly by mechanical shearing. Within 24 h, the process-less cells had settled on the dish and only a few began to extend short processes. Despite the lack of extensive process formation, at least 60% of the cells were electrically excitable. Both of these experiments indicate that process outgrowth, *per se*, is not responsible or necessary for the development of the action potential mechanism.

The action potentials in the PC12 cells were readily abolished by tetrodotoxin (3×10^{-7} M), an agent known to block Na-action potentials (Fig. 1*c*). In one experiment, action potentials were elicited from 11 of 12 impaled cells in control medium (mean $V_m = -27$ mV), none of 15 cells in TTX-containing medium (mean $V_m = -30$ mV), and 7 of 14 cells in control medium after washout of TTX (mean $V_m = -27$ mV). There were no residual regenerative potentials seen in TTX, as are seen in murine C1300 neuroblastoma cells[9]. Action potentials could also be recorded in PC12 cells in Ca-free solution (Fig. 1*d*). The TTX and Ca-free solution data indicate that the PC12 action potential is generated predominantly by a Na mechanism. Similar results have been found for action potentials of sympathetic neurones[10]. We did not attempt to elicit action potentials in TTX and high Ca, so we cannot exclude a small Ca component to the action potential. Our data indicate that any such component would not contribute a significant amount to the action potential at physiological ionic concentrations.

When NGF is added to PC12 cultures, cell division stops within 4–5 d and the cells begin to put out processes after this time. By approximately 16–18 d most of the cells have processes. We have recorded from cells in sister PC12 cultures after varying days in NGF. Figure 2 shows the percentage of electrically excitable cells in cultures treated with NGF for up to 3 weeks. Because of injury to many cells, each point may be an underestimate, but there is no indication on the basis of the mean resting potentials that NGF protected the cells from impalement injury. The time course for the development of electrical excitability roughly parallels that of the outgrowth of processes[2].

Response to iontophoretically applied acetylcholine

Acetylcholine (ACh) is the depolarising neurotransmitter for both adrenal chromaffin cells and sympathetic ganglion cells[8,10,11]. We tested PC12 cells for responsiveness to ACh in two ways. Microelectrodes filled with 1 M ACh (50–200 MΩ) were positioned just outside the cell to be impaled (within 5 μm). ACh was kept from diffusing out of the electrode by a 5–8 nA negative holding current (although careful testing with sensitive cells revealed that holding currents of 0–2 nA were sufficient for most electrodes used). When a stable impalement was obtained, discrete pulses of ejecting current between 0.5 and 50 ms duration and up to 50 nA were applied to the ACh electrode. The sensitivity of the PC12 cell was then determined as the amount of depolarisation produced by a given current (expressed as mV nC^{-1}). A second test for ACh sensitivity involved switching the holding current from negative (holding) to positive (ejecting; never more than 9 nA) for several seconds. Sensitive cells depolarised within 1–2 s. Less sensitive cells usually responded almost as fast but less intensely.

PC12 cells grown with NGF for more than 2 weeks responded

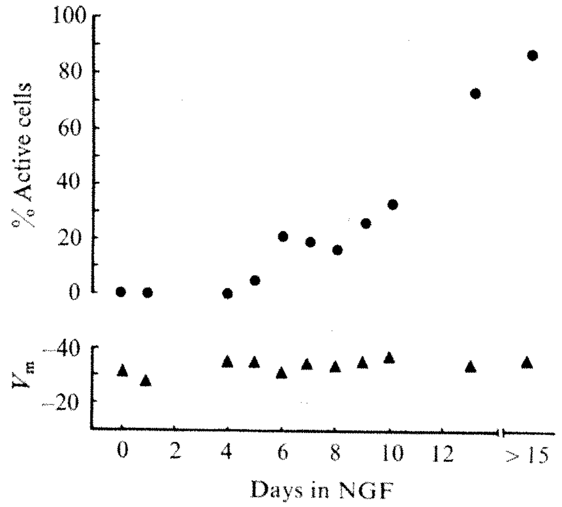

Fig. 2 Percentage of cells in PC12 cultures with action potentials (upper ordinate) and mean membrane potentials (lower ordinate) of these cells (see text) against the no. of days of exposure to NGF. Numbers of impalements for each point were: No NGF, 111; 1 d, 12; 4 d, 22; 5 d, 48; 6 d, 60; 7 d, 77; 8 d, 70; 9 d, 22; 10 d, 18; 13 d, 15; more than 15 d, 42. The standard errors of the mean membrane potentials were between 1 and 3 mV. Note increase in excitability from day 5 to day 13 with no significant change in mean membrane potential.

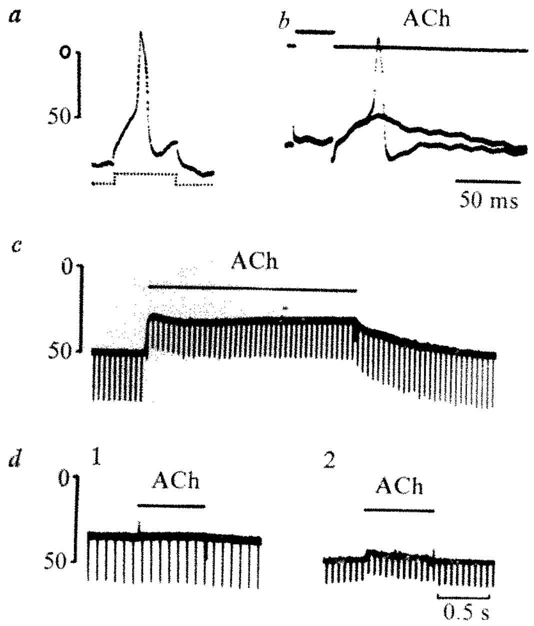

Fig. 3 Response of PC12 cells to iontophoretically applied ACh. *a*, Intracellularly applied depolarising current (dotted line) reaches threshold and triggers an action potential in a NGF-treated PC12 cell. *b*, Two superimposed traces of membrane potential responses to iontophoresis of ACh (14 nA, 30 ms iontophoretic current indicated in top line) on to same cell as in (*a*). Induced depolarisation reached threshold in one trace and triggered an action potential. *c*, Penwriter record of membrane potential of NGF-treated PC12 cell during steady iontophoretic application of ACh ($I = 6.5$ nA). Constant current hyperpolarising pulses were applied to the cell to indicate decrease in input resistance during ACh application. *d*, Penwriter records of ACh responses in two PC12 cells from cultures not treated with NGF. Note no response in (1) ($I = 8.5$ nA) and very small depolarisation in (2) ($I = 6.5$ nA) associated with barely detectable change in input resistance. These records were typical of responses in NGF-treated and non-treated cells. Time calibration in (*d*) applies to *c* and *d*.

to short pulses of current by producing short latency depolarisations which could trigger action potentials (Fig. 3*a*, *b*). Sensitivity was as high as 100 mV nC^{-1}. The ACh response was also associated with an increased membrane conductance (Fig. 3*c*). The ACh-induced depolarisation became larger with background hyperpolarisation and smaller with background depolarisation of the cell. In the above respects, the response resembles the ACh response seen in chromaffin cells[7,11,12] and sympathetic ganglion cells[10]. Prolonged iontophoretic currents produced depolarisations between 4 and 44 mV (mean of 20 cells, 17 mV) which were also associated with an increased membrane conductance. There were very few cells in the NGF-treated cultures which were unresponsive to ACh. No cells produced hyperpolarising responses.

No cells in the NGF-untreated cultures could be depolarised with short pulses of ACh—even with pulses up to 50 nA in intensity and 50 ms in duration and after hyperpolarisation of the cells to between −50 and −80 mV. Sensitivities as low as 1 mV nC^{-1} would have been detectable. But, PC12 cells grown without NGF could be depolarised by prolonged iontophoretic currents of ACh, but to a much lesser extent than the NGF-treated cells (Fig. 3*d*). The maximum response to several seconds of 8 nA current was 6 mV (mean of nine cells, 4 mV).

The responses to short pulses of ACh in the NGF-treated cells was of short latency (< 5 ms). *d*-Tubocurarine at 10^{-5} M added to the perfusing medium abolished all responses to short pulses of iontophoretic current and substantially reduced the ACh response to prolonged currents. These properties suggest that the ACh response was mediated through nicotinic receptors. This would correlate with the demonstration that noradrenaline can be released from PC12 cells by nicotinic agents[5].

Does inhibition of cell division induce differentiation?

The above data indicate that NGF treatment of PC12 cells profoundly affects their electrical excitability and chemosensitivity. One question raised by these results is whether such changes are merely a consequence of the arrest of cell division which takes place in the presence of NGF, or whether they are due to a more specific action of the factor. To test the former possibility, PC12 cells were treated with the anti-mitotic agents cytosine arabinoside (ara-C, 0.5–3 μM) and bromodeoxyuridine (BUdR, 3 μM) for up to several weeks. While both drugs produced a stable population of non-dividing cells, they did not stimulate fibre outgrowth. Physiological experiments revealed that a low proportion (10%) of such cells could generate active responses (Table 1). These responses were, however, much smaller in magnitude than those displayed by the NGF-treated cells. Furthermore, the ACh sensitivity of the division-arrested cells was comparable with non-NGF-treated rather than NGF-treated cells. Interestingly, cells treated with ara-C maintained their ability to respond to NGF. Such cells extended processes and displayed electrical excitability and ACh sensitivity properties comparable with those of NGF-treated control cells (Table 1).

The above findings suggest that the arrest of cell proliferation alone is not sufficient to account for the effects of NGF on the physiological behaviour of PC12 cells. Such changes are likely to be part of the overall constellation of differentiative events which are triggered by the factor.

Comparison with neuroblastoma cells

Another widely used model culture system with which PC12 cells might be compared are murine C1300 neuroblastoma cells. In appropriate conditions, the latter may also exhibit neuronal features, such as neurotransmitter synthesis, ACh sensitivity and electrical excitability[1]. The action potentials of neuroblastoma cells have both Na and Ca components[9]; those of PC12 cells involve predominantly Na. Neuroblastoma cells show hyperpolarising as well as depolarising responses to ACh[1,13] while the ACh responses of PC12 cells are exclusively depolarising. In addition, PC12 cells have been found thus far to differentiate only in response to NGF (ref. 2), while murine C1300 neuroblastoma cells can be induced to stop dividing and differentiate by a number of different agents but not by NGF (ref. 1). In this regard, the PC12 cells may be analogous to human neuroblastoma cells, which have also been shown to extend processes in response to NGF treatment[14–16]. PC12 cells are also analogous to cultured human pheochromocytoma cells which also respond to NGF and are electrically excitable[16,17].

PC12 line as a model system

The morphology and catecholaminergic properties of NGF-treated PC12 cultures have suggested that the overall effect of the factor on such cells is to promote their differentiation into sympathetic-like neurones[2,4,16]. The present findings regarding the emergence of electrical excitability and a high level of ACh sensitivity strongly support this contention.

Pheochromocytoma tumour cells are generally considered to be neoplastic counterparts of normal adrenal medullary chromaffin cells. The NGF-treated PC12 cells, in common with both human pheochromocytomas[17] and normal chromaffin cells[7], can synthesise and store catecholamines, are able to generate action potentials and can exhibit relatively high levels of ACh sensitivity. These properties are also shared with sympathetic neurones. In fact, sympathetic neurones and adrenal chromaffin cells are both of neural crest origin and are both derived from a common stem cell[18]. The low degree of physiological development and the ability to take on neuronal properties suggest that the PC12 cells not treated with NGF may possess the characteristics and pluripotency of a primitive, less differentiated common progenitor. If the analogy of PC12 cells to such a precursor holds, then this, in turn, supports the suggestion[3,19] that NGF, in addition to its documented effects on post-mitotic cells, has a critical role in promoting the development of normal sympathicoblasts into mature, electrically excitable neurones. Thus, the PC12 line may be a unique model system in which to study the differentiation of neuroblasts into neurones as well as for clarifying the role, mechanisms, and consequences of action of NGF on its target cells.

Our results also suggest that the PC12 line should be useful for physiological studies. In particular, it may be feasible to use the PC12 line to obtain biochemical correlates of the development of electrical excitability and chemosensitivity. Seen from a somewhat different orientation, our findings suggest that PC12 cells may be useful to study certain cell–cell interactions. For example, since NGF-treated PC12 cells are highly sensitive to ACh, they should be capable of receiving cholinergic synaptic inputs. Furthermore, not only do PC12 synthesise, store and release catecholamine neurotransmitters, but, like cultured sympathetic neurones[20], they may also synthesise, store and release ACh (ref. 21). Thus, PC12 cells may be capable of forming synapses with a variety of target cells as well as with one another.

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Large number hypothesis and continuous creation cosmologies

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The large number hypothesis and the condition that general relativity is satisfied in Einstein units, allows a family of cosmological models, two of which are the Dirac model without creation and the more recent Dirac model with multiplicative creation. The new models have multiplicative creation, a cosmological scale factor $S(t) \propto t^m$, *and are spatially flat; a multiplicative steady state model also satisfies the hypotheses. How these models affect the temperature of the Earth and the cosmological deceleration parameter is important.*

GRAVITATIONAL and cosmological physics yields three parameters that characterise the macroscopic structure of the Universe—the Hubble constant H, the constant of gravity G, and the average matter density ρ. These parameters are measured in units of mass length and time, a unit independent measure is obtained by comparing them to microscopic parameters of the same dimensions. If m is the mass of an electron, $-e$ its charge, and c the velocity of light, such unit independent measures are

$$\frac{mc^3}{e^2H} \simeq 6\times10^{40}, \quad \frac{e^2}{Gm^2} \simeq 4\times10^{42}, \quad \frac{m^4c^6}{e^6\rho} \simeq 4\times10^{40} \qquad (1)$$

These ratios are pure numbers, characteristic of the Universe. They are exceedingly large and approximately equal (the equality could be improved by using other microscopic units).

There are two points of view on these coincidences. One is that physics contains arbitrary parameters. The Universe could be different—it just happens that in our world, these parameters have their observed values. One argument is[1,2] that for human life to evolve and ask why the Universe is as it is, these parameters must have values close to those observed; the Universe could be radically different but we would not be here. I feel these arguments are not convincing either at the technical or philosophical level. The second view is that these coincidences are due to some interconnection between microscopic and macroscopic physics. Eddington[3] sought to develop a theory of such a casual interconnection, Dirac's[4,5] approach is to acknowledge that although at present we do not understand this relationship, we can proceed by assuming that for all time

$$\frac{mc^3}{e^2H} \simeq \frac{e^2}{Gm^2} \simeq \frac{m^4c^6}{e^6\rho} \qquad (2)$$

and further, that any other large number of the order of 10^{40} is approximately equal to these parameters. Since the first parameter is essentially the age of the Universe in microscopic units all these parameters vary in time but remain equal to each other. This is the large number hypothesis.

Cosmological models and the large number hypothesis

The application of this hypothesis to cosmology restricts the class of possible world models described in atomic units by the Robertson–Walker metric

$$ds^2 = dt^2 - \frac{S^2(t)}{c^2}\left(\frac{dr^2}{1-kr^2/R_0^{\,2}} + r^2\,d\theta^2 + r^2\sin^2\theta\,d\varphi^2\right) \qquad (3)$$

where $S(t)$ is the scale factor, R_0 a constant length and

$k=0, +1$, or -1. This metric has at least two characteristic lengths, that are, in principal, empirically determinable (by using atomic clocks and sending light signals between three non colinear co-moving cosmological observers). They are the Hubble length L_H and the radius of curvature of the space t = constant L_S defined as

$$L_H = \frac{c}{H} = \frac{cS}{\dot{S}}\ ;\ L_S = \frac{R_0 S}{k},\ k \neq 0 \qquad (4)$$

Both these lengths are very large and vary as $S(t)$ varies. The large number hypothesis requires that they be proportional to each other, which requires

$$k \neq 0,\ S = S_0 t + S_1\ ;\quad k = 0,\ S(t) \propto t^m \text{ or } S(t) \propto \exp(t/t_0) \quad (5)$$

The first result is obvious, since if $k \neq 0$, $\dot{S}$ is constant. As the Universe is observed to be expanding, we can further impose $S_0 \neq 0$ and so S_1 can be absorbed into the zero of time measurement. There are then only two such models, the Milne model[4] $k = -1$, $S(t) \propto t$, and the recent Dirac[5] model $k = +1$, $S(t) \propto t$. If $k = 0$, L_S is not a measurable parameter, however, $S(t)$ is not an arbitrary function of t; to see this we invoke Milne's dimensional argument[6]. Another measurable parameter is the deceleration parameter

$$q = -\frac{S\ddot{S}}{\dot{S}^2} = q(t) \qquad (6)$$

This is a pure number. If q varies in time, there must exist a constant t_0 of dimension's time such that $q = q(t/t_0)$ The theory now contains a constant t_0 and a variable $S/\dot{S}$, but the large number hypothesis requires them to be proportional to each other, so either $S/\dot{S} \alpha\ t_0$, $S \propto \exp(t/t_0)$ or $q = S\ddot{S}/\dot{S}^2$ = constant. The second alternative gives the first solution if $q = -1$, otherwise $S(t) \propto t^m$ where $q = (1-m)/m$.

Turning now to equation (2) the first and second terms give $G \propto H$ so that in general G varies in time as measured in atomic units. The first and third conditions give $\rho \propto H$, but as the Universe expands, the number of particles in a co-moving volume $N_0 \propto \rho S^3$, hence $N_0 \propto HS^3$ which is general varies with time. Particles must, therefore, be created or annihilated, the exception being the original Dirac model $S(t) \propto t^{\frac{1}{3}}$. In Table 1 we list the Robertson–Walker models satisfying the large number hypothesis.

Table 1 Cosmological models satisfying the large number hypothesis*

Model	k	$S(t)$	H	G	N_0
Milne	-1	t	$1/t$	$1/t$	t^2
Dirac	$+1$	t	$1/t$	$1/t$	t^2
Steady State	0	$\exp(t/t_0)$	$1/t_0$	const	$\exp(3t/t_0)$
Power Law	0	t^m	m/t	$1/t$	t^{3m-1}
Original Dirac	0	$t^{\frac{1}{3}}$	$1/3t$	$1/t$	const

*The three models $S(t) = 1$, $k = 0, +1, -1$, are ruled out by the condition $H \neq 0$.

Field equations in Einstein units

To advance any further, it is necessary to impose some additional constraints on the theory. First, where is the matter created? In the standard steady state theory[7,8], the newly created matter is supposed to be created *uniformly* throughout space and time. Dirac has recently proposed that creation is multiplicative[5]—that is, new matter is created in proportion to existing matter; we shall accept this hypothesis. The empirical success of general relativity further led Dirac to hypothesise that this theory must remain valid for macroscopic physics, due allowance being made for the creation of matter, and was led to deduce the new model described above, $S(t) \propto t$, $N_0 \propto t^2$, we shall also retain this hypothesis; however, it hardly restricts the possible cosmological solutions, only the Milne model does not satisfy this constraint.

Since general relativity is to describe macroscopic gravitational physics, we work in Einstein units in which the macroscopic mass mN_0, the constant of gravity G, and the velocity of light c are constant. All we are doing here is to define as our unit of mass as (say) the mass of the sun, our unit of time is defined by a gravitational clock (say) the orbital period of the Earth, and the unit of length is defined from the unit of time and a defined constant velocity of light. In this set of macroscopic units, Einstein's equations

$$R_{ij} - \frac{1}{2} g_{ij} R + \Lambda g_{ij} = \frac{8\pi G}{c^2} T_{ij} \qquad (7)$$

are valid. However, since matter is being created, the number of elementary particles in the sun varies in time, so, too, do the mass m and charge e in these gravitational units, subject to the condition that mN_0 is constant.

If τ and ρ are time and distance in Einstein units, then if the Robertson-Walker metric

$$ds_E^2 = d\tau^2 - \frac{S^2(\tau)}{c^2}\left(\frac{d\rho^2}{1-k\rho^2/R_0^2} + \rho^2 d\theta^2 + \rho^2 \sin^2\theta d\varphi^2\right) \qquad (8)$$

is introduced into the field equation (7), we obtain the usual family of cosmological models. However, most of these are incompatible with the large number hypothesis, introducing two or more cosmological lengths that are not proportional to each other. The only admissible solutions are those that satisfy equation (5) of which there are just two.

Einstein models:

$$S = 1,\ k = +1,\ \Lambda = \frac{1}{R_0^2} = \frac{4\pi G\rho}{c^2},\ \rho\text{, constant} \qquad (9)$$

Einstein–de Sitter model:

$$S \propto \tau^{2/3},\ k = 0,\ \Lambda = 0,\ \rho S^3 = \rho_0\ ,\ \text{a constant} \qquad (10)$$

Large number hypothesis in Einstein units

The large number hypothesis shown by equation (2) is, of course, independent of any system of units. In Einstein units with G, c and ρ_0 constant this requires

$$H \propto \frac{m^3}{e^4}\ ,\quad \frac{e^8}{m^6} \propto S^3,\ \text{hence } H \propto \frac{1}{S^{3/2}} \qquad (11)$$

The Hubble constant H is not S'/S (the dash denotes differentiation w.r.t. τ). It is defined, and measured, as the constant of proportionality in the red shift luminosity distance relation. Besides the usual contribution due to expansion in τ time, there is a further contribution due to the change in frequency of a particular atomic transition as e and m vary in τ time, this frequency

$$\nu \propto \frac{m}{e^2} \propto \left(\frac{1}{m^2(\tau)S^3(\tau)}\right)^{1/4} \qquad (12)$$

The ratio of the frequency of radiation from a given transition in a distant source at time τ_1, to the frequency of radiation from the same transition as an observer is, therefore

$$\frac{\mu_1}{\mu_0} = \frac{S(\tau_0)}{S(\tau_1)}\left(\frac{m^2(\tau_1)\,S^3(\tau_1)}{m^2(\tau_0)\,S^3(\tau_0)}\right)^{1/4} \tag{13}$$

The source is at co-moving Einstein coordinate $(\rho_1\tau_1)$, the receiver at $(0, \tau_0)$, since the signal propogates along null geodesics we have

$$\int_0^{\rho^1} \frac{d\rho}{1-k\rho^2/R_0^2} = \int \frac{c\,d\tau}{S(\tau)}, \quad \tau_1 = \tau_0 - \rho\,\frac{S(\tau_0)}{c} + \ldots \tag{14}$$

and hence equation (13) becomes

$$\frac{\nu_1}{\nu_0} = 1 - \left(\frac{1}{4}\frac{S'}{S} - \frac{1}{2}\frac{m'}{m}\right)\rho_1\frac{S(\tau_0)}{c} + \ldots \tag{15}$$

Now ρ_1 is the coordinate distance, not the luminosity distance l_1, to leading order, however $l_1 = \rho_1 S(\tau_0)c$; to second order this relation is not valid due to the change in luminosity of the source in τ time, the change of $S(\tau)$, the curvature of spaces $\tau = \text{constant}$ and the creation of photons in the beam of radiation as it travels from the distant source to the receiver. These effects will be considered below when estimating the deceleration parameter for these models. To leading order the red shift luminosity distance relation is

$$cz = Hl, \; H = \left(\frac{1}{4}\frac{S'}{S} - \frac{1}{2}\frac{m'}{m}\right) \tag{16}$$

We can now combine this result for the Hubble constant in Einstein units with the result (11) $H \propto S^{-3/2}$ to obtain the two solutions that satisfy the large number hypothesis and Einstein's equation in Einstein units
Einstein Universe:

$$S = \text{constant},\; H = \text{constant},\; m \propto \exp(-\tau/\tau_0)$$
$$N_0 \propto \exp(\tau/\tau_0),\; e \propto \exp(3\tau/4\tau_0) \tag{17}$$

Einstein–de Sitter Universe:

$$S \propto \tau^{2/3},\; H \propto \tau^{-1},\; m \propto \tau^{-n},\; N_0 \propto \tau^{n},\; e \propto \tau^{(1-3n)/4} \tag{18}$$

where $n \neq -1/3$ but is otherwise arbitrary ($n = 1/3$ gives $H = 0$).

The cosmological models in atomic units

Having obtained the equations (17) and (18) in Einstein units we can transform them back to the more familiar atomic units (AMU) in which e, m, c are constant but G and ρ_0 are not. (We could have scaled Einstein's equations in Einstein units to obtain their equivalent form in atomic units by the conformal transformation $g_{ij} \rightarrow \beta g_{ij}{}^*$, and worked throughout in AMU: the results would, of course, be the same. However, we prefer to use Einstein as this emphasises the assumptions, and simplifies the analysis.) The AMU of length and time

$$l_A \propto \frac{e^2}{m} \propto (m^2 S^3(\tau))^{1/4} \tag{19}$$

varies in Einstein units but is by definition constant in AMU. All lengths and times in Einstein units must, therefore, be scaled by this factor to obtain their values in AMU, so that if t is atomic time
Einstein model:

$$dt \propto (m^2S^3)^{-1/4}d\tau \propto \exp(\tau/\tau_0)\,d\tau \tag{20}$$

Einstein–de Sitter model:

$$dt \propto (m^2S^3)^{-1/4}d\tau \propto \tau^{(n-1)/2}d\tau \tag{21}$$

These integrate to give
Einstein model:

$$t \propto \exp(\tau/\tau_0) \tag{22}$$

Einstein–de Sitter model:

$$t \propto \tau^{(n+1)/2} \; n \neq -1 \tag{23}$$

$$t \propto \ln\tau/\tau_0 \; n = -1 \tag{24}$$

Similarly, the metric in AMU is obtained by scaling the metric in Einstein units

$$ds_A{}^2 = \frac{ds_E{}^2}{(m^2S^3)^{1/2}}$$

yielding
Einstein model:

$$ds_A^2 = \exp(\tau/\tau_0)\left(d\tau^2 - \frac{1}{c^2}\left(\frac{d\rho^2}{1-\rho^2/R_0{}^2}\right) + \right.$$
$$\left. + \; (\rho^2 d\theta^2 + \rho^2 \sin^2\theta\, d\varphi^2)\right) \tag{25}$$

Einstein–de Sitter model:

$$ds_A^2 = \tau^{n-1}\left(d\tau^2 - \frac{1}{c^2}\left(\frac{\tau}{\tau_0}\right)^{4/3}(d\rho^2 + \rho^2 d\theta^2 + \rho^2 \sin^2\theta\, d\varphi^2)\right) \tag{26}$$

which gives the three solutions: the Dirac Creation Model (DC), the power law models (PL) and the Steady State theory with multiplicative creation (MSS)

$$\text{DC: } ds_A^2 = dt^2 - \frac{1}{c^2}\left(\frac{t}{t_0}\right)^2\left(\frac{dr^2}{1-r^2/R_0{}^2} + r^2 d\theta^2 + r^2 \sin^2\theta\, d\varphi^2\right),$$
$$N_0 \propto t^2,\; G \propto 1/t \tag{27}$$

$$\text{PL: } ds_A^2 = dt^2 - \frac{1}{c^2}\left(\frac{t}{t_0}\right)^{2m}(dr^2 + r^2\theta^2 + r^2 \sin^2\theta\, d\varphi^2),$$
$$N_0 \propto t^{3m-1},\; G \propto 1/t \tag{28}$$

$$\text{MSS: } ds_A^2 = dt^2 - \frac{1}{c^2}\exp(2t/t_0)\,(dr^2 + r^2 d\theta^2 + r^2 \sin^2\theta\, d\varphi^2),$$
$$N_0 \propto \exp(3t/t_0),\; G = \text{const} \tag{29}$$

Of the possible solutions listed in Table 1, only the Milne model $S \propto t$, $k = -1$ is excluded. The condition that the large number hypothesis be satisfied and that Einstein's equations be satisfied in Einstein units (G c, ρ_0 constant) does not lead to a unique cosmological model, but to two special cases, the Dirac creation cosmology and the steady state cosmology with multiplicative creation, and to a family of power law models $S(t) \propto t^m$. Indeed, even the Dirac creation cosmology is not unique since R_0 in the equation (27) is arbitrary. Only by introducing a theory of the creation process can we expect to narrow down the range of possible models, for example, if there has been no creation or annihilation of matter since the 'origin' of the Universe, the only viable model is the original Dirac model without creation $S(t) \propto t^{1/3}$. However, I see no metaphysical attraction in such a hypothesis, and in the absence of any empirical evidence, there is no way to differentiate between the models.

The black body temperature of the Earth

For all the models derived here, we can calculate the variation of the thermal equilibrium temperature of the Earth during its lifetime. This is not, however, necessarily the actual thermal history of the Earth since many factors, such as atmospheric composition, and albedo need to be considered, but it should give some indication of the consequences of these models for the terrestrial environment. The flux of radiation falling on the Earth varies for several reasons, (1) the Sun's luminosity is sensitively dependent on the value of G, and the mass of the Sun, (2) the Earth's orbit spirals outwards (or inwards) as the mass of the Sun and Earth and G vary, (3) the Sun evolves chemically. We shall neglect (3), at constant mass and G this corresponds to an increase in solar luminosity of about 35%.

The luminosity of a star (L) of about solar mass depends on its mass M and the constant of gravity G approximately as $L \propto G^7M^5$. Working in AMU we find that the equilibrium temperature

$$T_e \propto \left(\frac{L}{r_A^2}\right)^{1/4} \propto \left(\frac{G^7N_0^5}{r_A^2}\right)^{1/4} \qquad (30)$$

where r_A is the radius of the Earth's orbit in AMU. Provided the newly created matter is created with the same velocity as its surroundings, thereby not exerting a drag on the Earth's orbit[9], the radius of that orbit is constant in Einstein units. Its value in atomic units is simply obtained by scaling by the factor $(m^2S^3)^{1/4}$ of equation (11); using the results in equations (27) to (29) gives

$$\text{DC: } T_e \propto t^{1/4},\ \text{PL: } T_e \propto t^{(9m-8)/4},\ \text{MSS: } T_e \propto \exp\left(\frac{9}{4}Ht\right) \qquad (31)$$

The estimated value of the Hubble constant is about 5×10^{-11} yr^{-1}, and the age of the Earth is about 5×10^9 yr, hence the ratio of equilibrium temperature today to when the Earth was formed is

$$\text{DC: } \frac{T_e}{T_0} \propto \left(\frac{4}{3}\right)^{1/4},\ \text{PL: } \frac{T_e}{T_0} \propto \left(\frac{4m}{4m-1}\right)^{(9m-8)/4},$$
$$\text{MSS: } \frac{T_e}{T_0} = \exp\left(\frac{9}{16}\right) \qquad (32)$$

Inasmuch as any conclusion can be drawn from climate studies, the flux of radiation falling on the Earth has increased. Indeed, if it was ever less than 90% of its present value, the Earth would now probably be completely ice covered[10]. This is a problem for standard theory which predicts a solar constant some 70% or so of the present value during the early history of the Earth[11]. If $m < 8/9$ the power law models yield a decreasing solar flux. (These models would not, however, explain the low neutrino flux since the evolutionary lifetime of a star $\propto L/M \propto t^{12m-11}$ so that such models would be more evolved than the standard model giving an increased solar neutrino flux.

The cosmological deceleration parameter

As the intrinsic luminosity of a star varies as the Universe evolves, so too will the intrinsic luminosity of a galaxy. This 'evolutionary effect' will manifest itself in the observed departures from the Hubble relation, through the deceleration parameter. While a detailed analysis requires a study of stellar and galactic evolution in these cosmological models, some idea of the observational consequences is obtained by assuming $L_{\text{galaxy}} \propto L_{\text{star}} \propto G^7M^5$.

Consider a galaxy at coordinate distance r_1, the energy per unit area received at the origin of coordinates at time t_0 (measured in atomic units) is[12]

$$P = \left(\frac{L(t_0)}{4\pi S^2(t_0)r_1^2}\ \frac{S^2(t_1)}{S^2(t_0)}\right)\left(\frac{N_0}{N_1}\right) \qquad (33)$$

where $L(t_1)$ is the luminosity of the source at the time of emission t_1, and the extra factor N_oN_1 is due to the multiplicative creation of photons in the beam on its journey from source to receiver. The coordinate distance r_1 satisfies

$$\int^{r_1} \frac{\mathrm{d}r}{1-kr^2/R_0^2} = \int_{t_1}^{t_0} \frac{c\,\mathrm{d}t}{S(t)},\ r_1 = \frac{zc}{S(t_0)}\left(1-\frac{1}{2}(q_0+1)z+\ldots\right. \qquad (34)$$

where z is the red shift of the source $(1+z)=S(t_0)/S(t_1)$, and $q_0=-S\ddot{S}/\dot{S}^2$ is the deceleration parameter at $t=t_0$. Equations (33) and (34) yield the magnitude redshift relation

$$m_s = -2.5\log\left(\frac{L(t_1)}{L(t_0)}\right) + 5\log z + 2.5\log(1-z)$$
$$-5\log H + \text{const} \qquad (35)$$

This relation is valid for all the models considered here, the value of m does not appear explicitly as the term

$$\frac{N_0}{N_1} = \left(\frac{S(t_0)}{S(t_1)}\right)^{(3m-1)/m} \simeq \left(1+\frac{3m-1}{m}z\ldots\right)$$
$$= (1+(2-q_0)z+\ldots \qquad (36)$$

and the terms involving q_0 exactly compensate. The same is true for the MSS model. Neglecting galactic evolution, all these models have an effective deceleration parameter $q_e = +2$, the particular characteristics of the model only enter through the evolution of the galaxy. If we assume that $L_{\text{galaxy}} \propto L_{\text{star}}$, then for the power law models

$$\frac{L(t_1)}{L(t_0)} = \left(\frac{t_1}{t_0}\right)^{(15m-12)} = \left(\frac{S(t_1)}{S(t_0)}\right)^{(15m-12/m)}\ 1+\frac{15m-12}{m}z+\ldots \qquad (37)$$

and again this result holds for the MSS model with $m\to\infty$. Including this estimate of the evolutionary effect, we find an effective deceleration parameter

$$q_e = 2-\left(\frac{15m-12}{m}\right) = \frac{12-13m}{m} \qquad (38)$$

For the DC model $q_e=-1$, for the original Dirac model without creation $m=1/3$, $q_e=23$, for the MSS model $q_e=-13$, for the constant Earth temperature model $m=8/9$, $q_e=0.5$. It would appear that there are models whose predictions are not incompatible with observation.

Conclusions

Assuming the validity of the large number hypothesis, multiplicative creation, and that general relativity is satisfied in Einstein units, we find a family of possible cosmological models. Two of these are the original Dirac model without creation and the new Dirac model with creation, but other solutions exist with $S(t) \propto t^m$, m arbitrary, and a multiplicative steady state theory. Further restrictions have to be imposed on the theory before this class of solutions can be narrowed down to one model.

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letters to nature

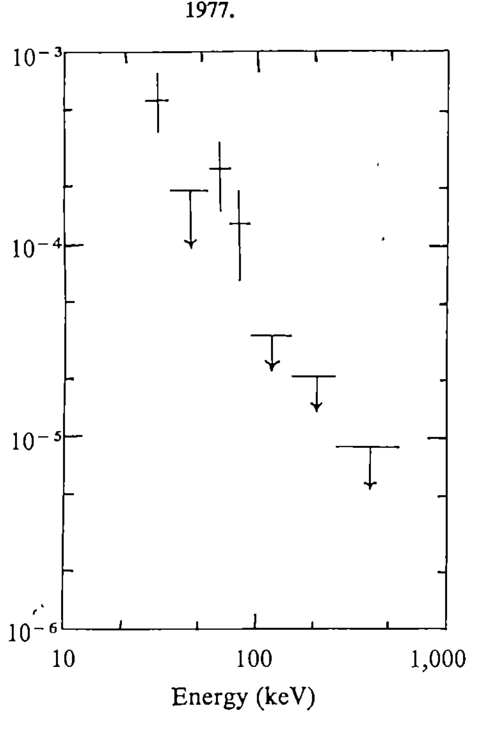
A line feature at 64 keV in the X-ray spectrum of Her X-1

TRUMPER and his colleagues[1] have reported observations which give evidence of a hard X-ray line feature in the spectrum of the pulsed flux of Her X-1. They suggest that this is cyclotron radiation from the poles of a neutron star. We discuss here some similar measurements made by the Imperial College Scintillation Telescope (ST) on Ariel 5 during a visit to the Hercules region, scheduled many months in advance of the observation. The binary system Her X-1 is known to emit X rays for at least 10 d in its 35-d cycle and our measurements were made during the ON period 3–13 February 1977.

The ST covers the energy range 26 keV to 1.2 MeV and consists of a CsI(Na), actively collimated detector crystal of 8 cm^2, offset from the satellite spin axis by 3°. Further details of the instrument and our observational technique can be found elsewhere[2]. We observed Her X-1 with the ST instrument in spectral mode for 2 d on the rising side of the light curve and 4 d on the falling side. This data has a time resolution of 8 min and thus we can only discuss the time averaged spectrum. Data from both epochs have been summed to obtain the best statistics and the resulting pulse height spectrum is shown in Fig. 1. All upper limits are 2 standard deviations (s.d.). It is clear from Fig. 1 that there is a significant flux excess in the 56–94 keV band.

To deconvolute this data from the detector response, a three-component spectrum was used consisting of a thermal bremsstrahlung component of $kT \sim 4$ keV, a line component at a variable energy, and a hard, power-law tail consistent with our upper limits. This gives the most conservative estimate for the significance of the line feature in the data. The relative intensities of the three components were varied, folded through a detector interaction matrix (as explained in ref. 2) and compared with the pulse height spectrum until a best fit was achieved. The fit was constrained to lie immediately below all the 2 s.d. upper limits. This spectrum was then used to deconvolute our data points and the result is shown in Fig. 2. Again all upper limits are 2 s.d. We believe that any spectrum consistent with the points shown in Fig. 2 would produce the pulse height spectrum observed. The fitted spectrum of Boldt *et al.*[3] from rocket flight data has also been shown in Fig. 2. Their absolute flux has been reduced by a factor of two to fit with our point at 30 keV. This fitting has no effect on the intensity of the line at 64 keV due to the steep nature of this part of the spectrum.

Fig. 1 The pulse height spectrum (converted to an energy scale) obtained from Her X-1 with the hard X-ray detector on Ariel 5 by combining data from the periods 4–6 and 9–13 February 1977.



Assuming the spectral feature evident in Fig. 2 is the result of a narrow line, we can set limits to its position by considering the detailed spectral response of our channels. This yields an energy of 64±6 keV at 1 s.d. Alternatively, by assuming a broader line centred near the mid-point of our channel, we can find an upper limit for its FWHM. This gives 16 keV at 1 s.d. Our estimate for the line intensity is 0.017 ± 0.007 photons cm^{-2} s^{-1} and this value is relatively insensitive to variations in the assumed underlying spectrum used for unfolding the results. The error is calculated for counting statistics only. Separate analysis of data from the rising and falling side of the X-ray light curve both yield evidence for the line feature.

The observations of February 1977 can be compared with a previous shorter observation[4] made by the same instrument in March 1975 during the early part of the ON phase. Identical analysis of these earlier data reveal a flux in the 26–34 keV band which is three times higher than the new flux. But no spectral feature is apparent at 64 keV and there is only a 2% probability that the line feature was also three times brighter than the new intensity. A line of intensity equal to the one reported here would not have been detectable due to the shorter observation time.

To explain the line, consider the usual model for X-ray emission from Her X-1 as being due to the infall of material near the surface of a neutron star after pushing its way down the polar funnels of the intense magnetic field. Correcting the line energy for gravitational red-shift (taking the origin of emission as 1.2×10^6 cm for a $1 M_\odot$ object) yields 73.1 keV as the initial energy. Classical cyclotron emission theory gives $h\nu_0 = (eHh/2\pi m_0 c) \times \sqrt{}(1-\beta^2)$ for the energy of first harmonic emission in a field H where β is the electron velocity expressed as a fraction of c and m_0 is its rest mass. This leads to a field strength estimate of $H = 7.3 \times 10^{12}$ G. At this field strength, however, the De Broglie wavelength of a 73 keV electron is four times its cyclotron radius and a relativistic, quantum mechanical treatment would seem more appropriate. Johnson and Lippmann[5] find total energy eigenvalue solution to Dirac's equation given by $E = \pm[c^2 p_{\parallel}{}^2 + m_0{}^2 c^4 + e\hbar cH (2n+s+1)]^{\frac{1}{2}}$, where $s = \pm 1$, the electron spin state, n is the principal quantum number and $p_{\parallel}$ is momentum parallel to **H**. Supposing that both parallel and anti parallel spin states arise while the material is falling into the polar funnels, $s = +1 \rightarrow s = -1$ transitions may be expected to occur, leading to energy release ΔE where $E_{\mathrm{i}}{}^2 - (E_{\mathrm{i}} - \Delta E)^2 = 2e\hbar cH$. E_{i}, the initial energy, is estimated as the sum of rest mass energy plus energy in the spin-field interaction plus thermal energy and we take the last of these contributions as 13 keV, based on a previous data fit of Carpenter *et al.*[4]. A value $H = 6.9 \times 10^{12}$ G results. Lodenquai *et al.*[6] find various allowed angular momentum states in a more complete, but non-relativistic treatment. Use of their work and the assumption of angular momentum transitions would lead to a field value roughly comparable with

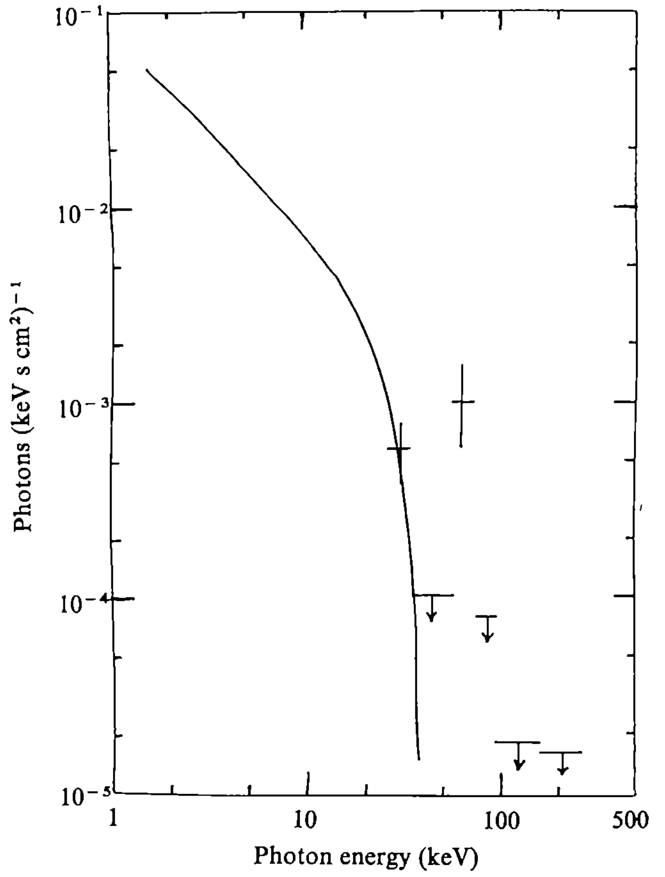


Fig. 2 The spectrum of Fig. 1 unfolded as described in the text. Also shown, as a continuous line, is the best fit spectrum from the proportional counter data of Boldt *et al.*[3] reduced in absolute intensity by a factor of two.

the above estimates. Note that a line arises naturally in the quantum mechanical treatment, rather than the wide spread implied by the $\sqrt{(1-\beta^2)}$ factor in the classical treatment. In fact, putting $\Delta E \propto \Delta H$ and assuming a dipole field implies an emitting region depth 8% of a neutron star radius in size if we take our observations of the line width. Also note that high electron kinetic energies and, by implication, X-ray continuum radiation at appreciable levels around 100 keV are not necessary. A classical treatment of the cyclotron self-absorption effect suggests that for the observed flux values, escape of the X rays considered is not a problem.

An alternative explanation in terms of radioactive decay of nuclides produced by explosive nucleosynthesis during neutron star formation more than 10^4 years ago is less promising. We use Clayton's[7] estimates of supernovae yields required to give the ^{60}Ni Solar System abundance, with 50% produced via the ^{60}Fe progenitor, which decays with a 3×10^5-yr half life. The ^{60m}Co decay to ground state within this chain is via a 58.6-keV ($\sim$ 50 keV after redshift) line, provided the internal conversion branch in atomic Co is inhibited by the degenerate gas conditions at the neutron star surface. A low Her X-1 distance estimate of 600 pc is then required to get $\sim 4\times10^{-5}$ cm^{-2} s^{-1} photons at the Earth, well short of the observed flux. Furthermore, a magnetospheric controlled screening process would need to be invoked to explain the occurrence of the line in the pulsed component and this would further decrease the predicted flux. No other process seems as likely as the one discussed. But a lack of temporal variation in the line flux would perhaps favour an explanation in terms of radioactivity, in which case the estimates for nuclide production from supernovae must be considerably revised.

A search for the equivalent proton cyclotron transition at 274 Å (after redshift) would be interesting. Radiation from such a line is likely to be highly perturbed by the surrounding plasma, however. If our explanation of the observations is correct, a new way of exploring pulsar magnetic fields is opened up.

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Relation between metallicity and multiplicity for solar type stars

It has been suggested that the ratio of heavy elements to hydrogen (metallicity) in a contracting interstellar cloud might affect the process of star formation. There is now statistical evidence for solar type stars that the metallicity of a forming stellar system is correlated with the multiplicity of the resultant system and may actually determine whether the system will be multiple or single.

The most direct and complete data on the multiplicity of solar type stars are given by Abt and Levy[1]. The δm_1 index[2,3] is thought to provide rather precise determinations of the relative metallicities of late F dwarfs and subgiants. Lindemann and Hauck[4] give photometric data sufficient to calculate δm_1 (β) for 87 of Abt and Levy's 135 stars, and $\delta m_1(b-y)$ for 27 more. Two of the stars must be omitted because they are too cool for β or $b-y$ to be reliable. Of the 112 remaining stars with available δm_1, Abt and Levy (their Table 2) describe 15 as having a 'probable' or 'possible' constant or variable velocity. Omitting these slightly uncertain cases from consideration, there are 97 stars with reliable values of δm_1 and definite statements whether their radial velocities are constant or not. The latter are interpreted as multiple systems while the former may be single stars, stars with low mass secondaries and/or extremely long periods, or close binaries viewed pole on.

The 97 stars divide into three categories of metallicity: (1) $\delta m_1 \leqslant 0.000$, the mean metallicity of the Hyades cluster stars or higher; (2) $0.000 < \delta m_1 \leqslant 0.030$, metallicities ranging from half that of the Hyades stars up to that of the Hyades stars; (3) $\delta m_1 > 0.030$, metallicities less than half that of the Hyades stars.

There are seven stars in the metal-rich group (1): six have a variable velocity (interpreted as multiple); one has a constant velocity and a companion with a common proper motion (CPM) and is interpreted here as a multiple system. None has a constant velocity and no known or suspected companion (interpreted as 'single?').

There are 74 stars in group (2): 35 have a variable velocity (multiple); nine have a constant velocity and a CPM companion or are known members of a visual binary system (multiple); 30 have constant velocities and no known or suspected companions (single?).

There are 16 stars in the moderately low metallicity group (3): one has a variable radial velocity (multiple); two have constant velocities and either has a CPM companion or is a known member of a visual binary (multiple); 13 have constant velocities and no known or suspected companions (single?).

Table 1 Multiplicity for three categories of metallicity

	$\delta m_1 < 0.000$		$0.000 < \delta m_1 < 0.030$		$\delta m_1 > 0.030$	
	Multiple	Single	Multiple	Single	Multiple	Single
High velocity	0	0	3	3	0	5
Low velocity	7	0	41	27	3	8
Combined	7	0	44	30	3	13

Eleven of the 97 stars are intermediate (not extreme) 'high velocity' stars with space motions in excess of 63 km s^{-1}. It has long been known that high velocity stars tend to be single and also that they tend to have low metallicity. Table 1 emphasises that a metallicity–multiplicity correlation exists within both the high velocity and low velocity groups. In other words, it is a phenomenon of both population 1 and population 2 stars. (It is important to determine whether the unresolved combination of the colours of two members of a close binary will produce an artificially small δm_1 leading to a false interpretation that the pair is richer in heavy elements than they actually are. Calculations of various combinations of the observed colours of apparently single, constant velocity stars suggest that this is not the case.) We conclude that the chemical composition of the star forming cloud is closely related to the probability of forming a multiple system.

There is new evidence from spectral quantification[5] and from spectral classification[6] that the Sun has the same metallicity as the mean of the Hyades cluster stars. This result, acceptance of a metallicity–multiplicity correlation, and the awareness that the internal scatter in the δm_1 values is about 0.01 magnitudes, suggest that all solar type stars of $\delta m_1 \lesssim 0.020$ (which is about $\frac{2}{3}$ the solar metallicity or greater) are multiple systems with the observed constant velocity systems being either systems viewed pole on or systems with low mass secondary stars or planets. This inference is consistent with Abt and Levy's conclusion that single stars are rare among low velocity stars.

The possibility also exists that all solar type systems of whatever metallicity form as multiples with the lower metallicity systems (including population 2) tending to have large mass ratios and being more likely to have planets as secondaries. Carried to the extreme, this speculation implies that the hypothetical population 2 planetary systems might have given rise to life more than 5,000 Myr before our Sun formed.

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Cosmic rays and and ancient catastrophes

A NUMBER of suggestions have been made[1–3] that encounters of the solar system with extra-solar phenomena in earlier epochs led to climatic and biological catastrophes. Most recently Whitten *et al.*[4] and Clark *et al.*[5] have drawn attention to the role of nearby supernovae (within ≃ 10 pc) from which catastrophic effects are expected (primarily the depletion of the atmospheric ozone layer and consequent transmission of damaging solar ultraviolet.) Whitten *et al.* estimate the probability of an encounter within a period of 10^8 yr to be 1–5% and Clark *et al.* calculate 50% for the same quantity. In this letter, we argue that it is likely that rare and very big solar flares, and their attendant cosmic-ray fluxes, will give even more dramatic effects. This idea is not new, in that Reid *et al.*[6] have drawn attention to the effect of solar flares on the evolution of life, but here we endeavour to estimate the likely frequency of solar flares of sufficient strength to have significant effects. Furthermore, we draw attention to the 'explosion' at the galactic centre postulated by some[7] to have occurred about 10^7 yr ago; if this actually took place then its effect too would have been important and exceeded likely supernova phenomena.

In view of the fact that the Sun is the nearest active astronomical object, it is clearly necessary to examine its activity characteristics in detail before going further afield. There is a wealth of data on solar flares and, for the past 35 yr, cosmic rays from the Sun have been recorded, with an increasing degree of completeness. Measurements have been made of particles at ground level, at a variety of altitudes and, recently, outside the atmosphere with satellites. In each case, data have been collected at various geomagnetic latitudes. We have summarised the published data (most of it relevant to high galactic latitudes where the geomagnetic cut off is small) in Fig. 1. The energy density received at the top of the atmosphere is taken as the relevant parameter (defined as integrated energy in the flare for particles above a threshold energy per unit area: erg cm^{-2}) and its frequency distribution is taken for two periods (1956–1960)[8] and (1961–1972)[9]. The fluxes for 1956–1960 refer to a threshold of 100 MeV and the corresponding fluxes for protons above 20 MeV will be higher by a factor of the order of 5. A comment is necessary about the magnitude of the threshold energy relevant to the present analysis. A prominent effect is the destruction of the ozone

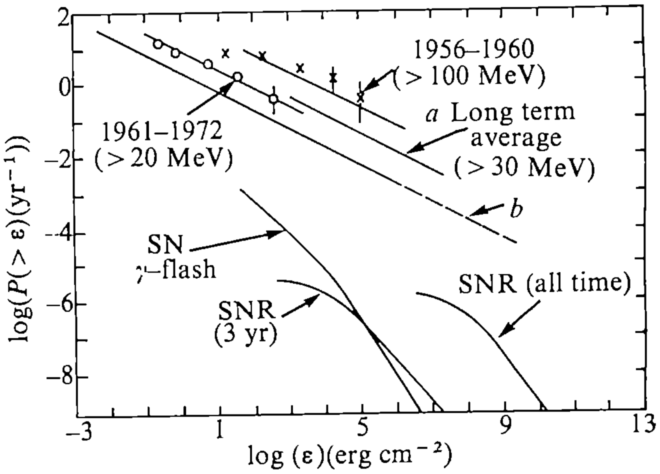

Fig. 1 Integral frequencies of solar cosmic-ray bursts at the Earth. The abscissa is the energy density in the burst and relates to the top of the atmosphere. Line *a* is a rough estimate of the long period average burst frequency (for energies above ~ 30 MeV) and is derived from measurements made on protons during the very active period 1956–1960 and during the most recent solar cycle 1961–1972 (the latter being of apparently rather average solar activity as judged by the mean sunspot numbers). Most of the particles under consideration normally arrive in the polar regions. Line *b* represents the frequency distribution when the burst energy density is averaged over the Earth's surface. SN γ-flash denotes the frequency distribution of energy deposition from the γ-ray flash from supernovae (using the result of Clark *et al.* 1977, for a supernova at 10 pc). SNR (3 yr) and SNR (all time) represent energy deposition over a 3-yr period, and integrated over the whole time, respectively, from protons when the Earth is immersed in a supernova remnant. *P* is probability and ε the energy density.

layer by NO produced after ionisation of the stratosphere[10] and the incident proton energy necessary to reach the appropriate levels in the stratosphere is $\simeq$ 30 MeV. Thus, an energy threshold of 20 MeV is only a little low for the necessary limit.

Two features are apparent in Fig. 1: there is a large difference in the absolute frequencies and the slopes of the variations are very similar in the two cases. The disparity in absolute frequencies relates to the difference in general solar activity in the two periods. The interval 1961–1972 covers the most recent solar maximum period together with the previous solar minimum and 1956–1960 comprises the corresponding previous solar maximum. The mean sunspot numbers are relevant; Allen[11] quotes $R_m = 110.6$ for the solar maximum in 1968.9 and $R_m = 201.3$ for 1957.9 to be compared with an overall average $R_m \simeq 112$ for the last 270 yr, the period over which reliable measurements have been made. There is, thus, some evidence for the frequency of bursts of solar cosmic-ray particles arriving at earth being strongly dependent on mean sunspot number. It is interesting that an analysis by Schove[12] suggests that only two periods, centred on 1372 and 1558 had similarly high sunspot numbers to 1956–1960. Thus, activity on the scale of 1956–1960, although rare is almost certainly not unique.

Remembering that the 1956–1960 data refer to energies above 100 MeV and that the contributions to $P(>\varepsilon)$, probability (> energy density), from the different cosmic ray flare magnitude ranges are additive we estimate that the very long term average for $P(>\varepsilon)$ and proton energies above 30 MeV is at least as high as line *a* in Fig. 1. There seems to be evidence for an emprical power law for $P > \varepsilon$ over seven orders of magnitude and we feel that this can probably be extended by at least several magnitudes more.

The region of interest with respect to supernovae is indicated in Fig. 1 by the line 'SN—γ flash'. The energy density of 10^6 erg cm^{-2}, corresponding to one event in 10^8 yr, is the value adopted by Clark *et al.* for γ rays from a supernova explosion at a distance of 10 pc and we have calculated the values for various distances to give the line. The interesting energy region considered by Clark *et al.* (10^8 erg cm^{-2}) is only a little way beyond the highest detected flare energy and extrapolation over this small energy range can surely be made. The extrapolation of line *a* itself is not immediately relevant, however, because the effective area over which the indicated energy density acts is not the full area of the earth, the reason being the shielding introduced by the Earth's magnetic field. As a very conservative figure we assume that the low energy particles in question arrive only at geomagnetic latitudes above 70°, where the average geomagnetic cut-off rigidity is only ~ 0.15 GV (it is permissible to use this value for a proton energy threshold of 20–100 MeV because the large cosmic-ray flares have rather flat energy spectra at energies below 200 MeV, or so). The operative area is ≈3% of the total. Using this factor the effective density averaged over the whole Earth is as shown in line *b*.

Another phenomenon is also relevant: the reversing, during past epochs, of the Earth's magnetic field[6]. During the period when the field is small, and the cutoff rigidity likewise, the flare particles will blanket the Earth completely. A very approximate analysis gives an effective average again not far from line *b* (we are indebted to C. J. Hatton and R. W. Girdler for discussions on these points).

Figure 1 also indicates that significant solar flare effects (similar to SN at 10 pc) will be expected about every 10^3 yr—much more frequently than expected for close supernovae—and, indeed, considerably stronger effects are likely on a time scale of 10^5 yr.

An important question is whether there is supporting evidence from other stars for the emission of energetic flares (and cosmic rays from them). Although in many solar flares the particle energy is only ≈1% of the total flare energy, in the largest cosmic ray flares, the particle energy seems to approximate to the optical energy[13] (≈ 10^{32} erg). Thus, the extrapolation is only to some 10^{34} erg or so in total energy in order to be well inside the SN region. There is little doubt that stellar flares do give cosmic-ray energies in this region. Lovell[14] has summarised the evidence for cosmic rays from M- and K-stars and shown that their flare energies are in the range 10^{33}–10^{37} erg. These flare stars are, of course, in different parts of the evolutionary path from the sun but Lovell makes a strong case for the same flare mechanism being operative in M-, K- and main sequence stars.

It is possible, in principle, to set limits to the frequencies of large optical and cosmic-ray solar-type flares from other data: the cosmic-ray flux in ancient times inferred from analysis of lunar samples, deep-sea sediments, etc. and the frequency of magnitude changes (from the associated optical flares) in main sequence stars. The situation with ancient cosmic-ray fluxes seems to be that the average flux has remained constant within a factor of 2 over the past 10^9 yr and the flux over the past 400 yr is within 10% the same as that over the past 4.10^5 yr (ref. 15). Although the bursts only last for the order of days or hours their intensities would be so high (10^4 times 1 yr galactic flux at 3×10^9 erg cm^{-2} for the linear extrapolation in Fig. 1) that they would contribute to long period averages. It is likely, therefore, that line *b* could continue to about 10^9 erg cm^{-2} without producing conflict with the ancient cosmic-ray measurements but above that it seems necessary to have a somewhat more rapid fall off.

There is, however, one set of measurements which do not fit into this pattern. These are from the studies of ^{10}Be and ^{26}Al in sea sediments[16,17] which seem to show a 10-fold increase in cosmic-ray intensity for a period of $\sim 10^6$ yr some 2.5×10^6 yr ago. Such an increase could be due to a small number of large bursts spaced over the period and if true would correspond to an extrapolation of line *b* without reduction.

The situation with respect to magnitude changes of main sequence stars is not very clear. It has been pointed out that the biggest flares (in 1956–1960) seemed to have high conversion efficiencies to cosmic rays so that the optical flare energies were, like the cosmic-ray energies, ~ 10^{32} erg. Presumably, for larger flares similar high efficiencies would result, so that, in order to achieve effects similar to SN at 10 pc, very infrequent optical flare energies of 'only' 10^{33}–10^{34} erg would be required. Flaring to this extent would seem to produce negligible magnitude changes in a reasonable sample of nearby stars. Continuing to a flare energy of ~ 3.10^{38} erg, a factor of $\sim 10^5$ beyond the SN threshold, if the average large optical flare lasts ~ 1 d only, 10^5 stars (out of 1.5×10^{11} stars in the Galaxy) should change by ~ 1 mag for 1 d yr^{-1}. It seems likely that such a low frequecy of flaring of this magnitude would not have been seen. Thus, the extrapolation of line *b* is allowable from astronomical data.

At this stage, it is obvious that the frequency of SN-equivalent cosmic-ray bursts is very high; some five orders of magnitude greater than calculated for SN at 10 pc. Thus, the effects postulated for SN would seem to occur with a very high frequency ($\simeq$ every 1,000 yr). In fact, we feel that much bigger bursts are probably required in order to have a really large effect on the ozone layer in the upper atmosphere[6] and a frequency of ~ 1 per 10^5 yr would seem more appropriate. Although this corresponds to a large extrapolation of line *b* there is a further large factor in hand because large bursts seem to be repetitive within the time constant (a few yr) for atmospheric ozone.

Reverting to supernovae, there is also the effect mentioned by Clark *et al.* of the increased cosmic-ray flux encountered when the solar system is inside a supernova remnant. The variation of cosmic ray intensity with time is the subject of conjecture; here we follow Clark *et al.* and adopt an increase over the local galactic intensity by 10^2 for the stage when the SNR radius is 10 pc. Assuming that the SNR merges into the I.S.M. when the radius is ~ 50 pc, the energy density over a 3-yr period (the approximate relaxation time of the atmosphere for O_3 production) follows the variation marked SNR (3 yr).

Allowance has been made for the lower efficiency of a galactic cosmic ray-type spectrum (of γ-rays) for depositing energy in the region of the atmosphere populated by O_3 (a factor of $\sim$ 25 down in energy density).

Also indicated in Fig. 1 is the energy density integrated over the whole of the SNR (SNR *p* (all time)). This variation would be relevant to those biological processes involving gradual reduction in quality over many generations.

It is interesting to estimate the likely radiological dose rate at the Earth's surface from the big solar flares. The galactic flux generates ~ 30 mR yr^{-1} at ground level (corresponding to $\sim 10^{-2}$ erg cm^{-2} s^{-1} of incident energy. Line *b* extrapolated indicates that a dose of 10^{11} erg cm^{-2}, corresponding to 10^4 R, would occur $\sim$ every 10^5 yr. Allowing for the eventual undoubted fall-off in $P(>\varepsilon)$ below the extrapolated line such a dose (lethal even to very primitive forms of life) would probably still occur more often than once every 10^8 yr. Such large flux densities do not necessarily indicate flares larger by many orders of magnitude compared with those observed in 1959. More likely would be situations where flares just a few orders bigger occurred at a time when a favourable (?) magnetic field geometry focused a significant fraction of the ouput onto the Earth.

Finally, attention is directed to a quite different phenomenon: the possibility of extremely energetic bursts from the galactic centre. Briefly, there is the possibility that the 3 kpc 'ring' is due to a radially symmetric explosive event in the galactic nucleus. With the ejection of $\sim 2 \times 10^8 M_\odot$ the initial energy is at least 3×10^{58} erg (ref. 7). On this model, the explosion occurred 12–13 × 10^6 yr ago (or earlier if there is oscillation of the ring). If the explosion resulted from the collapse of a very massive object then γ-ray emission would be very likely and, according to Colgate[18], the fraction of energy in this form would be large. Extrapolating from the value for less massive objects we take $f \sim 10^{-3}$ giving $\simeq 10^{55}$ erg in γ-rays. If $\sim 10\%$ of galaxies were to give bursts of this type then γ-ray bursts would occur at about the observed frequency.

With the γ-flash from the galactic centre the energy density at the Earth is $\sim 10^9$ erg cm^{-2}, a value that again exceeds the flux from local supernovae.

In conclusion, in addition to close supernovae, solar flares and explosions at the galactic centre should be borne in mind when considering possible catastrophes experienced by the Earth in its history. A more detailed study of solar and stellar flare data and ancient records of solar activity would appear to be profitable.

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Tunguska's comet and non-thermal ^{14}C production in the atmosphere

THE dramatic explosion on 30 June 1908 over Tunguska, Siberia continues to generate a wealth of literature on the supposed mysterious nature of the phenomenon. Hughes[1] recently summarised the generally convincing case for the hypothesis, first put forward by Whipple[2] and Astapovich[3], that a comet collided with the Earth's atmosphere. Opposition to this theory and consequent support for less conventional hypotheses involving black holes[4], almost critical masses of extraterrestrial fissionable material[5], anti-matter bodies[6] and alien spacecraft[7] have been based substantially on the non-observance of the comet before impact, the explanation of height of the explosion above the Earth's surface, the composition of the glassy spherules found at Tunguska and finally, and of most importance, the apparent occurrence of nuclear phenomena in the explosion as indicated by the subsequent enhancement of radiocarbon in the atmosphere. In the following, we discuss the first and last of these points which are clearly the most important.

Ben-Menahem[8] re-analysed the old seismograms of the Tunguska event and deduced the parameters of the original explosion by comparing the old data with contemporary records of the seismic and acoustic effects of recent nuclear air explosions. He concluded that the main explosion had an energy of $(5 \pm 1) \times 10^{23}$ erg and occurred at a height of 8.5 km. The size of the incident body can be estimated by assuming that its original kinetic energy was entirely converted into explosive energy. Krinov[9] found the geocentric velocity to be between 28 and 50 km s^{-1}, Levin[10] gave a geocentric velocity of 47 km s^{-1}. This latter value gives the body a mass of 5 × 10 g. Assuming the body is a cometary nucleus, of density 1 14 g cm^{-3} (ref. 11) its equivalent diameter is found to be 40 m. The absolute visual magnitude, m_0 of a comet of mass M (its magnitude 1 AU from both the Sun and Earth) is given by[12]

$$\log_{10}(M \text{ in g}) \simeq 21 - 0.4\, m_0 \qquad (1)$$

so the Tunguska comet had $m_0 = +26$.

The apparent magnitude, m of the comet when it is a distance r AU from the Sun and Δ AU from the Earth is given by[12]

$$m = m_0 + 5 \log_{10} \Delta + 10.5 \log_{10} r \qquad (2)$$

The two equations given above are mean, rule of thumb relationships obtained by considering the observed parameters of a great many comets. Some comets are notorious for departing from expected behaviour but in the absence of any observations of the Tunguska comet we can only assume that it was one of the more well behaved members of its species and obeyed the equations given.

Table 1 Apparent magnitude, m and time to impact t of the comet as a function of its distance from the Earth

Δ(AU)	r(AU)	m	t(min)
0 002	1.0	12 4	104
0 001	1 0	10 9	51
0.0005	1 0	9 4	24
0.0002	1.0	7 4	8
0.0001	1 0	5.9	3

Values of m for certain specific values of Δ are given in Table 1. Also given in Table 1 is the time t taken by the comet to travel, at 47 km s^{-1}, from a point Δ AU away from the Earth to the Earth's surface At the turn of the century comets were usually discovered at magnitudes around +11. The Tunguska comet is only brighter than this value for about 1 h before it impacts with the Earth, ample reason for it not being seen on its earthward path. Fesenkov[13] finds that, for all possible cometary orbits calculated from the fireball trajectory, the angular distance between the comet and the Sun is small. When the comet encountered the Earth it would be coming from a point in the dawn sky comparatively close to the Sun and would thus be most difficult to detect and observe. The orbital configuration is similar to Comet Mrkos which was only detected after having rounded the Sun and travelled beyond the Earth's orbit.

It is most difficult to calculate the expected flux of these very small cometary nuclei to the Earth's surface because of the lack of relevant observational data. Vsekhevyatskii[14] gives cumulative number data for comets with $1 < \mathrm{mag}_0 < 14$ A wanton extrapolation of Whipple's[15] interpretation of this data gives a value of 7×10^8 for the number of comets brighter than $\mathrm{mag}_0 = +26$ which pass within 1.2 AU of the Sun within 100 yr. Using Everhart's[16] calculations the chance of such a comet striking the Earth is about 5×10^{-2} in 100 yr; so one on average should hit the Earth every 2,000 yr.

Comparing the ablation and deceleration of the cometary nucleus with that of a meteoroid forming a meteor train it can crudely be assumed that one atom (or molecule) of the incident body is ablated (albeit with a high energy) when it is struck by an atom (or molecule) of the atmosphere. The cometary nucleus thus explodes when it has collided with a mass of air equal to its own mass. Assume that the atmosphere has a density, ρ, at a height h of $\rho = \rho_0 \exp(-h/H)$ where ρ_0 is the density at ground level and H is the scale height (~ 7 km). The cometary nucleus (radius r_c, density ρ_c moving on a trajectory inclined at an angle χ to the vertical will intersect a mass M_c in descending to height h_1, where

$$M_a = \pi r_c^{\,2} \sec\chi\, \rho_0 \int_{h_1}^{\infty} \exp(-h/H)\, \mathrm{d}h \tag{3}$$

M_a is equal to the comet mass $(4\pi\rho_c r_c^{\,3}/3)$ at the explosion height $h_{\bullet}$ given by

$$h_{\bullet} = H \log_e \left(\frac{3 \sec\chi\, \rho_0 H}{4\pi r_o \rho_c} \right) \tag{4}$$

Putting $h_e = 8.5$ km, $H = 7.1$ km, $\rho_0 = 10^{-3}\ \mathrm{g\,cm^{-3}}$, $r_o = 2.1 \times 10^3$ cm and $\rho_c = 1.14\ \mathrm{g\,cm^{-3}}$ gives $\chi = 1.5°$. Fesenkov[13] concluded that χ was between 65° and 70° and probably larger, Levin[10] calculated χ to be 82°. The low value calculated above is probably due to the initial assumption of a one to one ratio between collision atoms and ablated atoms. The formation of an aircap around the nucleus in the lower, denser, regions of the atmosphere will lessen the ablation and enable it to traverse a longer atmospheric path and thus have a higher value of χ.

Magnetite and silicate globules, 80–100 μm in size, were recovered from the soil near the explosion site Detailed analysis of these revealed small amounts of Co and Ni as well as traces of Cu and Ge[17] and this has been offered as partial proof of the 'alien spacecraft' hypothesis. It must be remembered, however, that these elements are most probably present in the dust of the cometary nucleus at their normal cosmic abundances. Their high values of ionisation potential vitiates against them showing up in cometary spectra.

One of the major stumbling blocks of the cometary impact Hypothesis is the ^{14}C anomaly that is said to have occurred in 1909. Cowan *et al.*[6] found that the ^{14}C activity was higher in that year than normal and deviated from the mean by over two standard deviations (but see ref. 18). Cowan *et al.*[6] manufactured this extra radiocarbon in the atmosphere by proposing that 1/7 of the Tunguska energy was due to anti-matter annihilation. Hunt *et al.*[5] produced this ^{14}C excess by a nuclear explosion at Tunguska. A sudden influx of ^{14}C into the Earth's atmosphere will be observed as a positive anomaly in the graph of radioactive CO_2 content as a function of time, this anomaly having a width (to half maximum values) of about 2.4 yr. This is because atmospheric CO_2 is continually being removed by plants in the biosphere, converted to humus and then returned to the atmosphere as this humus decays; 5% is absorbed each year in this way. CO_2 also dissolves into the oceans, rainfall helping considerably, and this removes about 20% yr^{-1} (see ref. 19). So 25% of the atmospheric CO_2 is being locked away each year, remains locked away for between 10 and 50 yr and is subsequently slowly returned by plant decay and oceanic evaporation.

Now, if all the kinetic energy of the comet (5×10^{23} erg) were converted into heating its constituent particles and an equal mass of the atmosphere (mean mass 12) the temperature obtained would be about 2×10^6 K. Since this is substantially sub-nuclear it has been argued that normal cometary impact could not have produced the observed radiocarbon. It is entirely fallacious to suppose that sub-nuclear temperatures cannot produce nuclear effects as can be seen by consideration of the solar flare phenomenon. In a flare, the mean temperature of the particles is certainly less than 2×10^7 K, that is an energy of order 10^{32} erg distributed among some 10^{16} g. Even at the low densities of the solar atmosphere, many associated high energy phenomena are seen to occur—the production of X and γ rays, MeV electrons and GeV nuclei[20]. The mechanism by which these are produced is still not properly understood but qualitatively may be attributed to stochastic acceleration of particles in the turbulent spectrum of plasma waves generated in the flaring atmospheres by the primary energy release. Although this process is magnetohydrodynamic in character the solar magnetic field need not play any direct part (other than as the source of energy) since the large particle velocities and the ionisation produced by any explosion can generate the necessary magnetohydrodynamic conditions. Similar results are, therefore, to be expected in the Earth's atmosphere following a cometary impact.

We can estimate the ^{14}C production in the Tunguska cometary impact by a rough scaling comparison with the flare results, without specifying the detailed process. The collisional production of energetic neutrons by the accelerated particles varies as $nNQV\tau$ where n is the atmosphere number density in the heated atmosphere, N the total number of particles in the heated volume, Q the neutron production cross section, V, the mean particle velocity, and τ the duration of the event. Thus if υ is the number of neutrons produced and the comet and flare characteristics are denoted by subscripts C and F then

$$\upsilon_c = \upsilon_F \frac{n_C N_C Q_C V_C \tau_C}{n_F N_F Q_F V_F \tau_F} \tag{5}$$

From the cometary mass of 5×10^{10} g we have $N_c \simeq 2 \times 10^{33}$ while the atmospheric density at 8.5 km gives $n_c \simeq 2 \times 10^{19}\ \mathrm{cm^{-3}}$ and for a flare $N_F \leq 10^{40}$. The density in the flare acceleration region is uncertain but is probably around $n_F \simeq 10^9\ \mathrm{cm^{-3}}$, and the typical flare duration is $\tau_F = 100$ s. Chupp *et al.*[21] have set an observational upper limit to the number of 20 meV neutrons formed even in a 2B flare as $\upsilon_F \leq 10^{25}$. Thus from equation (5) we have

$$\upsilon_C \simeq 3 \times 10^{26} \left(\frac{Q_F V_C}{Q_F V_F} \right) \tau_0 \tag{6}$$

The factor in parentheses in equation (6) is uncertain because the detailed process of neutron formation is unknown Certainly the typical nuclear collision speed V_c in a cometary impact explosion may be less than that in a flare (V_F) since the explosion temperature driving the plasma process is lower. On the other hand, the neutrons proposed by Cowan *et al.*[6] for radiocarbon formation are only of about 10 MeV energy so Q_C/Q_F may be substantially larger than unity. If, therefore, we estimate the whole parenthetic term in equation (6) as unity then we expect $3 \times 10^{26}\ \tau_C$ neutrons to be formed in a Tunguska-type cometary impact. By comparison Cowan *et al.*[6] estimate that about 10^{27} neutrons are required to explain the radiocarbon data. Thus, if the necessary hot plasma conditions were sustained in the Earth's atmosphere for at least 3 s (a very plausible figure compared with the duration of enduring meteor trails, overdense diffusion times, etc.) then the required neutron formation would probably ensue by non-thermal plasma processes.

We conclude, therefore, that within the uncertainties of the effect of chemical composition on collective plasma acceleration processes and on collisional neutron production, the number of neutrons expected in the Tunguska impact, as scaled from solar flares, is in remarkably good agreement with the radiocarbon data requirements. Also the low mass of the comet (5×10^{10} g) coupled with its extreme faintness ($\text{mag}_0 = +26$), and its position in the dawn sky, just before impact makes its prior discovery extremely unlikely. These points strongly support the suggestion that the Tunguska explosion was caused by an impacting small comet and that nothing more exotic need be invoked.

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Antarctic topography from balloons

ANTARCTIC ice surface elevation maps are far from being complete, due to the difficulties in applying regular surveying and mapping techniques in Antarctica. A new wealth of ice sheet surface topography data is now available, from many balloons, floating at an altitude of about 12.5 km, which traversed the Antarctic during its 1975 summer. These balloons were part of 411 constant density balloons launched in the Southern Hemisphere during the Tropical Wind, Energy conversion and Reference Level Experiment (TWERLE)[1]. Each balloon carries three sensors: radio altimeter[2], pressure sensor[3] and ambient temperature sensor. The balloon transmits data once per minute, as long as its solar panel is sufficiently illuminated[4]. The data are received by the Random Access Measuring System (RAMS)[5] on board the NIMBUS-6 satellite, whenever the balloon is within line of sight.

The near Polar orbit of NIMBUS-6, and the continuous summer daylight combined to allow continuous coverage of TWERLE balloons, south of 66°S, during December 1975 and January 1976. Continuous coverage means 13–14 passes per balloon in 24 h. The longest pass is an overhead one, which lasts more than 15 min, hence the data consists of 15 transmissions, or less, per pass. In addition to recording the balloon's sensors data, RAMS also measures the received carrier frequency. The Doppler history of up to 15 transmissions during an overpass yields the balloon position[6]. Two consecutive passes can produce the wind information.

The average balloon life during TWERLE was slightly more than two months, with some balloons lasting more than a year. The launching strategy and the general circulation at balloon height caused a typical daily concentration of about 40 balloons south of 45 °S, during December 1975–January 1976. Many of these ballons traversed the Antarctic Continent.

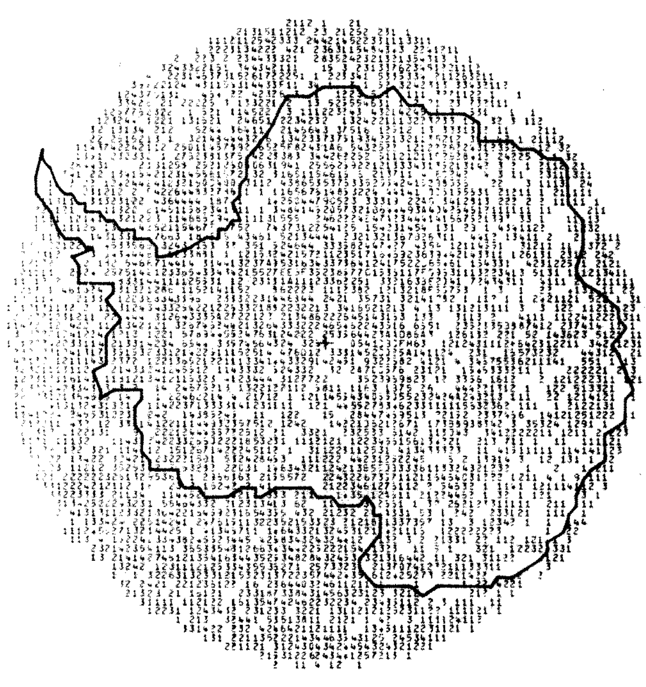

Fig. 1 TWERLE balloons coverage over Antarctica 1 November 1975–19 March 1976. Each symbol indicates the number of measurements over a square 55×55 km (1 = 1,. . . . 0 = 10, A = 11, B = 12,. . . . .). The data boundary corresponds to 65 °S, and 0° longitude is up.

The extent of Antartic coverage by the TWERLE balloons is summarised in Fig. 1. This gives the total coverage from all the balloons during the period 1 November 1975–19 March 1976. Each symbol on the map indicates the total number of measurements over a square of 55×55 km. A measurement is the average of up to 15 balloon transmissions, 1 min apart, recorded during the satellite overpass. As Fig. 1 shows there is almost complete coverage over Antarctica, with many squares being traversed more than once.

The radio altimeter data over Antarctica resembles overwater data, as far as standard deviation of the 15 altitude readings per pass. This small standard deviation is due to the flatness of the ice cover. The ice flatness further contributes to the measurement accuracy by reducing the adverse importance of position error, which is specified at 5 km.

To obtain the surface elevation from the altimeter readings it is necessary to know the balloon's altitude above sea level (a.s.l.). Constant altitude could not be assumed because (1) the altitude of a constant density level is not constant, and (2) the balloon equilibrium density changes following diurnal cycles. What can be assumed constant, for several hours at least, is the altitude a.s.l. of a pressure level along the balloon trajectory. This is the result of the quasi-geostropic nature of the wind. In other words, the balloon, which follows the wind, is moving parallel to the isobars. It can be concluded that the balloon altitude a.s.l. could be calculated if the altitude of a reference pressure surface above the balloon subtrack is known once a day. 150 mbar was chosen as this reference pressure, because it is the standard radiosonde level nearest to the balloon flight level.

The relation between the surface elvation, the altitude of the 150 mbar pressure surface and the altimeter reading is

$$E = Z(150\ \text{mbar}) - (P + P_{\text{corr}} - 150)\,(0.2T + 53.7) + \\ + (75 \cos^2\Phi - 15) - h \qquad (1)$$

where E is the surface elevation a.s.l. in metres, $Z(150\ \text{mbar})$ is the altitude of the 150 mbar surface in geopotential metres, P is the pressure sensor reading in mbar, P_{corr} is the pressure sensor correction in mbar, T is the ambient temperature reading in °C, Φ is the latitude and h is the altimeter reading in metres.

The second term on the right hand side is the altitude difference between the balloon's pressure level and the 150-mbar pressure level, using a linear approximation to the hydrostatic relation. The third term is an approximation to the difference in the balloon altitudes when given in units of geopotential metres and geometric metres. P_{corr} is the difference between true pressure and the pressure sensor reading. Once in altitude, the TWERLE pressure sensor is very stable. Erroneous prelaunch calibration, however, and very long term drift may cause a bias error of up to 3 mbar. This bias error can be removed by several comparisons with 150-mbar synoptic maps, when the balloon flies over water in an area well covered by radiosonde data, such as Australia and New Zealand.

The altitude of the 150-mbar level above the balloon subtrack, for use in equation 1, should also come from radiosonde data. The radiosonde network in Antarctica is, however, too sparse to produce good synoptic maps. To our help come all the other TWERLE balloons in the area. Balloons over water provide both the geopotential height of the pressure surface and the wind. Balloons over land are used for wind only. Figure 2 is one of the synoptic maps drawn for the analysis of a test case balloon no. 766. On that day, there were only nine radiosonde stations south of 45 °S which reported the wind and/or the geopotential height of the 150-mbar level, at the mandatory time of 1200 GMT. There were, however, 32 more TWERLE balloons south of 45 °S reporting the same information.

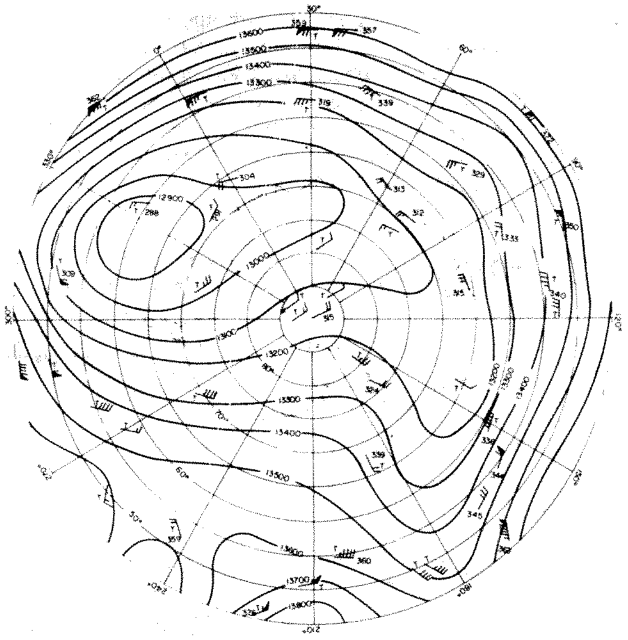

Fig. 2 Synoptic map of the 150 mbar pressure surface at 1200 GMT, 11 December 1975. Constructed from nine radiosonde reports and 32 TWERLE balloons (marked with 'T'). The balloon marked 'X' is no. 766, whose full trajectory appears in Fig. 3.

The first balloon to be analysed during its Antarctic traverse was balloon no. 766. Its trajectory from 3 December 1975 till 14 December 1975 is plotted in Fig. 3. Next to each position, the elevation of the Antarctic continent calculated using Equation 1 is given. P_{corr} for balloon no. 766 was checked six times during the 100 d preceding the traverse, and was found to be 1.7 mbar. Six synoptic maps, similar to Fig. 2, were plotted for the traverse period.

The estimated error budget in the calculated elevation is as follows: pressure surface: ± 30 m, altimeter: ± 10 m, pressure sensor: ± 10 m, position error of 5 km at a slope of 2 m km^{-1}: ± 10 m. Adding all these yields a worst-case error of ± 60 m. This accuracy is comparable to accuracies of ± 30 m obtained during aircraft radio echo soundings[7], and to accuracies of ± 50 m obtained in over-snow traverses using the two barometric altimeters method[8].

There are several absolute check points available for the elevation data in Fig. 3. First there are three measurements of the sea surface elevation before crossing the shore line at the beginning of the traverse. These exhibit a maximum error of 31 m. Then there are the sea surface measurements at the end of the traverse, which exhibit maximum error of 18 m. A relative check is available at the poleward most crossover in the trajectory. On 8 December 1975 an elevation of 2,038 m was obtained when the balloon passed over position 85° 97′S, 324° 25′E. Three days later, an elevation of 2,042 m was obtained for 85° 98′S, 324° 29′E.

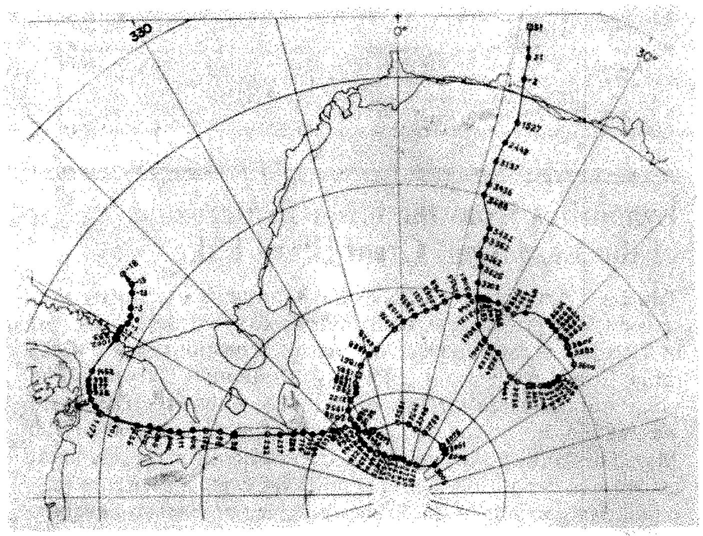

Fig. 3 The traverse of TWERLE balloon no. 766 over Antarctica, 3–14 December 1975. The Antarctic surface elevation at the balloon subtrack was calculated using equation (1), and is given in metres. The first known position on a GMT day is marked by a square.

Another indication of the accuracy is available from a preliminary comparison with the Radio Echo Sounding Map of East Antarctica[7], performed on 33 well distributed localities, measured from five different balloons. The average error was +7 m (balloon data being higher), and the standard deviation of the differences was 36 m. Whenever the balloon subpoint did not coincide with an elevation contour line of the radio echo map, a linear interpolation between the two neighbouring contour lines was used. In five out of the 33 comparisons, the differences were larger than 60 m (the largest being −83 m), however, it should be noted that the sum of the two accuracies is ±90 m. It can be concluded that the accuracy of the data, and the extensive coverage promise significant additions to the Antarctic ice maps. (The data already analysed is available from Nadav Levanon, in tabulated form.)

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Evaporites from the lower Proterozoic of the East Arm, Great Slave Lake

THE East Arm of Great Slave Lake contains excellently preserved lower Proterozoic sedimentary rocks that were deposited in fluviatile, coastal and shallow marine conditions. These rocks range in age from about 2,400 Myr to about 1,600 Myr. The polar wander curve for this period[1] shows that the East Arm was within 30° of the equator during the period 1,900–1,700 Myr. The sediments deposited during this period show abundant evidence of deposition in arid conditions, especially in that they contain indications of the former presence of extensive evaporites. Evidence of former evaporites is lacking in those sequences deposited apparently beyond 30° from the palaeoequator. Permian to modern marine evaporites are generally confined to ±30° of latitude. It is proposed, therefore, that climatic (and by possibly atmospheric and hydrospheric) conditions were grossly similar in the lower Proterozoic to those of today. These deposits pre-date similar sequences in the MacArthur River area by some 300 Myr (ref. 2). They predate the supposed initiation of sulphate evaporites by 1,000 Myr (ref. 3) and clearly negate Cloud's various hypotheses about the evolution of the Earth's atmosphere and hydrosphere.

The East Arm of Great Slave Lake is a graben within which there was intermittent sedimentation and igneous activity from 2,500 Myr ago to 1,300 Myr ago[4]. Three major groups have been defined[5]. (1) The Wilson Island Group. A group of shallow water sedimentary rocks and volcanics older than 2,200 Myr; (2) The Great Slave Supergroup, deposited between about 2,200 Myr and 1,790 Myr; (3) The Et-Then Group, deposited between ~ 1,700 Myr and 1,600 Myr.

The Wilson Island Group has been metamorphosed and deformed, although locally there are well preserved, unmetamorphosed sections. The other groups are unmetamorphosed and beautifully preserved. Throughout much of the lower Proterozoic deposition was in shallow water and fluviatile, tidal-flat and shallow marine sediments are abundant (Table 1).

The polar wander path for North America[1] indicates that the East Arm within 30° of the equator during the deposition of the middle and late Great Slave Supergroup (from about 1,900 Myr to about 1,700 Myr). The bulk of Permian-modern marine evaporites has been essentially confined to ±30° of latitude[6]. The depositional environments in the East Arm indicate that ideal conditions for evaporite formation were frequent. The palaeomagnetic data indicate that we could expect evaporites in the sequence between 1,900 Myr ago and 1,700 Myr ago. In Table 1, we show those formations that contain evidence for evaporites and we document the evidence below.

The oldest unit in which there is evidence for evaporites is the Kluziai Formation. In the Snowdrift area, there are nodules of slightly ferroan dolomite within fine-grained, well-sorted sandstones. The nodules are usually restricted to certain horizons. They are 1–2.5 cm in diameter and usually consist of a single poikilotopic dolomite crystal. Similar nodules occur in the top of the Hornby Channel Formation in the same area but are not well preserved. The nodules are similar to ones found in the Zambian Copperbelt sandstones, and Annels[7] has shown how carbonate has replaced plates of anhydrite and gypsum which are still preserved in places in these rocks. It is proposed that the nodules in the Kluziai Formation have replaced original anhydrite.

Additional evidence for evaporites in the Sosan Group comes from the Akaitcho River Formation on Seton Island where

Table 1 Evidence for the presence of evaporites in the East Arm, Great Slave Lake

		Group	Formation	Lithology	Deposition environment	Evidence for evaporites
	1200					
		Et-Then	Preble	Sandstones	Fluviatile	
			Murky	Conglomerates	Alluvial Fan	
Great Slave Supergroup	1795	Diorite Intrusion				
		Christie Bay	Pearson	Basalts and argillites	Alluvial Plain?	
			Portage/Inlet	Red shale, ssts and slts	Sabkha?	Halite and gypsum casts
			Tochatwi	Sandstones	Marine to fluviatile	Casts of anhydrite nodules?
			Stark	Dolomites and Red Mdsts	Tidal arid coast	Halite casts and broken beds
			Hearne	Limestones	Shallow marine	
			Wildbread	Limestones	Shallow marine	
			Pekanatui Point	Limestones and shales	'Deep' marine	
		Pethei	Blanchet	Turbiditic sandstones	'Deep' marine	
			McLean	Red/brn mdsts and Lmsts	'Deep' marine	
			Utsingi	Limestones	Supratidal	Broken beds, crinkled laminae
			Taltheilei	Dolomites	Shallow marine	
			Douglas Peninsula	Red siltstone and mdst	Sabkha	Gypsum casts, broken beds
			Charlton Bay	Green shales with concretions	Shallow marine	
		Kahochella	McLeod Bay	Red shales with concretions	Intertidal	Gypsum casts
			Gibraltar	Red shales	Supratidal, arid coast	Gypsum casts
	1872		Seton	Alkali volcanics		Gypsum casts
			Akaitcho River	Red sandstones and slts	Coastal, Deltaic	Gypsum casts
		Sosan	Kluziai	Sandstones	Fluviatile/marine	Dolomite nodules after anhydrite?
			Duhamel	Dolomites	Shallow marine	
			Hornby Channel	Pebbly sandstones	Fluviatile to shallow marine	Dolomite nodules after anhydrite
	2200					
		Union Island		Basic volcanics, Dolomites and Red and black shales	Shallow marine	
		Wilson Island		Volcanics, Quartzites, Dolomites and siltstones		
	>2485	Basement				

Olade and Morton[8] described pseudomorphs after gypsum from red siltstones and shales. These sediments are overlain by volcanics of the Seton Formation, which have been dated on Seton Island at 1,872 Myr (ref. 9). In the Akaitcho River Formation at Reliance, there are small (0.5 mm) nodules of length slow chalcedony (quartzine), which may indicate replaced evaporites[10]. It is interesting that these occur in well-sorted glauconitic sandstones. The glauconite occurs as pellets which have cracks infilled with carbonate—a feature that has been ascribed to desiccation[11]. In these two areas, the Formation was deposited in shallow marine, perhaps intertidal conditions. On the north shore, at Talthelei Narrows, red mudstones of the Akaitcho River Formation contain beds with haematite pellets and carbonate nodules as well as casts after gypsum. These beds are interpreted as pedogenic horizons and probably were formed in a sabkha environment.

Pseudomorphs after gypsum are also common in the Kahochella Group. They occur in the upper parts of the Gibralter Formation in intertidal red mudstones with stromatolites, intraformational conglomerates and pelletoid ironstones[12]. Casts after gypsum have been described from tuffs of the Seton Formation, again associated with pedogenic horizons, and from red shales with carbonate concretions in the McLeod Formation near Reliance. The Douglas Peninsula Formation contains similar casts in red fissile mudstones with carbonate or haematite/jasper laminae. Hoffman[12] has tentatively proposed a lacustrine origin for these rocks which probably formed in a sabkha environment. During the remaining Pethei Group times shallow-water sediments were only deposited on a northern platform. Of the platformal sequences, the Utsingi Formation of stromatolitic limestones and dolomites has been tentatively described as a supratidal sabkha deposit[12]. We have observed evaporite breccia and contorted laminae within the unit at Reliance. Both features resemble closely those in the Purbeck Formation of Dorset, where the latter has been cited as part of the evidence for the former presence of evaporites[13].

On Isui Island, there is a sequence of fissile, red, calcareous mudstones containing thin laminae of red-stained carbonate and calcite pseudomorphs after anhydrite. The sequence is in fault contact with other units, but closely resembles the Douglas Peninsula Formation with which it is tentatively correlated[17]. The laminae are disturbed by small scale cross-lamination and scours, but also by crinkle folds (1 cm amplitude) which probably formed while the rock was still plastic. There are plugs of brecciated, banded drusy calcite up to 3 m across cutting these sediments. The plugs contain clasts of red dolomitic mudstone. Plugs of oligomictic breccia with no calcite cement are also seen to cross-cut bedding. The drusy calcite contains streaky layers of red calcareous mudstone. Rosettes and euhedral crystals of barite occur in bands in these layers of calcareous mudstone and overgrow the drusy calcite. These features closely resemble the upper zone of the cap rock of a salt dome[14] except that they are on a far smaller scale. They are, however, also similar to structures produced by small scale movements over anhydrite beds[15]. There is no evidence for halite in nearby rocks, but pseudomorphs after anhydrite are abundant. We, therefore, suggest that these plugs were perturbations in the top of an anhydrite bed that has subsequently been calcitised.

In Christie Bay Group times, the polarity of platform and basin broke down, and once again shallow marine to arid coastal conditions prevailed over much of the East Arm. The best evidence of former evaporites comes from the Stark Formation which consists of dolomites, red mudstones and siltstones. Stromatolite mounds, crinkled algal laminae, flat pebble conglomerates, wave ripple marks, and loading and shrinkage cracks all point to an intertidal to supratidal origin[12,16]. The laminated dolomites are often highly brecciated, and there are various types of breccia. In places, almost the entire thickness of the formation (in excess of 1,000 feet) is disrupted to a megabreccia. Elsewhere small patches of breccia occur amidst undisturbed strata. The breccias were attributed to gravity tectonics around rising diorite laccoliths,[4,12] an hypothesis rejected by Badham[18] who proposed that they were analogous to evaporite breccias elsewhere and the result of collapse after solution of evaporites. More recently Hoffman *et al.*[19] have concurred with this view and have proposed an elegant model.

The Stark Formation retains evidence, other than the breccias, for the former presence of evaporites. The dolomites contain vugs wholly or partially filled with ferroan dolomite and younger calcite. The vugs are up to 2.5 cm long and compaction has occurred around them. They are confined to pyritic, stromatolitic dolomite and are most common in the base of algal mounds. They disrupt original laminae and are associated with shrinkage cracks. The association is similar to that in the Middle Jurassic of Scotland[2]. The vugs are similar to the elongate fenestrae of the medium laminoid fenestral fabric and are assumed to have once contained gypsum[21]. The red mudstones contain abundant and well developed pseudomorphs after halite. These are now usually filled with dolomite, but in the aureoles of the diorite laccoliths feldspar is the replacing mineral. It is suggested that the Stark formation originally contained thick sequences of evaporites, most of which were removed in solution with concomitant collapse of overlying strata. Some of the breccias and deformation within the Upper Christie Bay Group may, however, be due to salt diapirism—a hypothesis which needs more detailed investigation.

The youngest evaporites in the East Arm occur in the Portage Inlet Formation. Pseudomorphs after halite are common in buff sandstones and shales, which commonly also contain desiccation cracks. Mudstones at the top of the Formation contain pseudomorphs after rosettes and single crystals of gypsum[12].

There is, therefore, abundant evidence for evaporites in the middle and late Great Slave Supergroup. Much, but by no means all, of this evidence is from pseudomorphs after gypsum. Gypsum may also form during the degradation of sulphides[22]. The nature of the sediments and the other evidence of evaporites suggest, however, that the gypsum was of evaporitic origin. Many of the East Arm evaporites were deposited on supratidal flats and sabkhas, an environment where similar sediments with evaporites are forming today. Evidence for former evaporites is found only in the middle and late Great Slave Supergroup and not in the Union Island and Lower Sosan Groups, when equally suitable depositional environments existed. This suggests that (1) the distribution of lower Proterozoic climatic belts (and thence the geographic distribution of evaporites) was broadly similar to that of today and (2) the palaeomagnetic data are correct.

Finally it is interesting to note evidence for the former presence of evaporites from rocks of the Belcher Group (in Hudson Bay) of similar age to the Upper Great Slave Supergroup[23]. This evidence supports the above conclusions.

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Polycyclic aromatic hydrocarbons in long-range transported aerosols

THE presence in Norway of long-range transported air pollutants from the European continent and England is well established. In particular, SO_2 and other inorganic compounds in precipitation have been studied[1]. Less attention has been paid to the organic constituents of the long-range transported pollutants. Several organic components have been identified in precipitation in Norway[2], including alkanes, polycyclic aromatic hydrocarbons (PAH), phtalic acid esters, fatty acid esters, fatty acids, and dicarboxylic acids. In addition some chemicals typically connected to industry and industrial products are present. Long-range transfer of PAH compounds, in particular, is of environmental interest since a number of such compounds cause cancer in animals and they are also suspected to be human carcinogens[3]. We report here preliminary results on the PAH-content of long-range transported and stationary air masses.

All samples were taken by the Norwegian Institute for Air Research at their sampling station at Birkenes in southern Norway. Airborne aerosols were collected by high-volume samplers on glassfibre filters. About 2,500 m^3 air was drawn through the filters per day, and the filters were changed every 24 h. The origin of the air masses transferring the pollutants has been indicated by trajectories which are calculated on the basis of meteorological data[4]. When the trajectories showed that consecutive filters were sampling air of the same origin, the filters were combined before analysis.

The air filters reported in this work represent two sets. The first set contains samples of air which had come from France/England (sample 1) and England/Scotland (sample 2). The other set contains samples of air coming from northern Norway (sample 3) or of stationary air from southern Norway (sample 4). The samples represent air sampled for 24, 48 and 72 h.

The analytical procedure used in this work is based on the method of Grimmer and Böhnke[5]. Gas chromatographic analysis was performed using a Carlo Erba Fractovap 2101 A gas chromatograph equipped with a 50 m × 0.34 mm i.d. glass capillary column coated with OV 1. The technique of splitless injection of the sample was used[6]. Retention times and peak areas were registered automatically by means of an electronic integrator. The PAH compounds were identified by comparing the chromatograms with those of the PAH-standards and the amounts were determined comparing the electronically integrated areas of each peak with those of the internal standards.

Results of the PAH analyses are given in Table 1 and a typical gas chromatogram is shown in Fig. 1. It is seen from the chromatogram that the system used is capable of separating critical isomers such as benzo(*a*)pyrene from benzo(*e*)pyrene and benzo(*a*)anthracene from chrysene and triphenylene. This separation is essential for characterising the potentially hazardous PAH components in environmental samples.

Table 1 shows that 20 different PAH compounds have been identified and determined. These include some of those reported to be carcinogenic[3]. It is also shown that the samples from western Europe contain about 20 times more PAH than the samples from northern Norway and the stationary air from southern Norway. Although these samples are taken over short periods only, and are not neces-

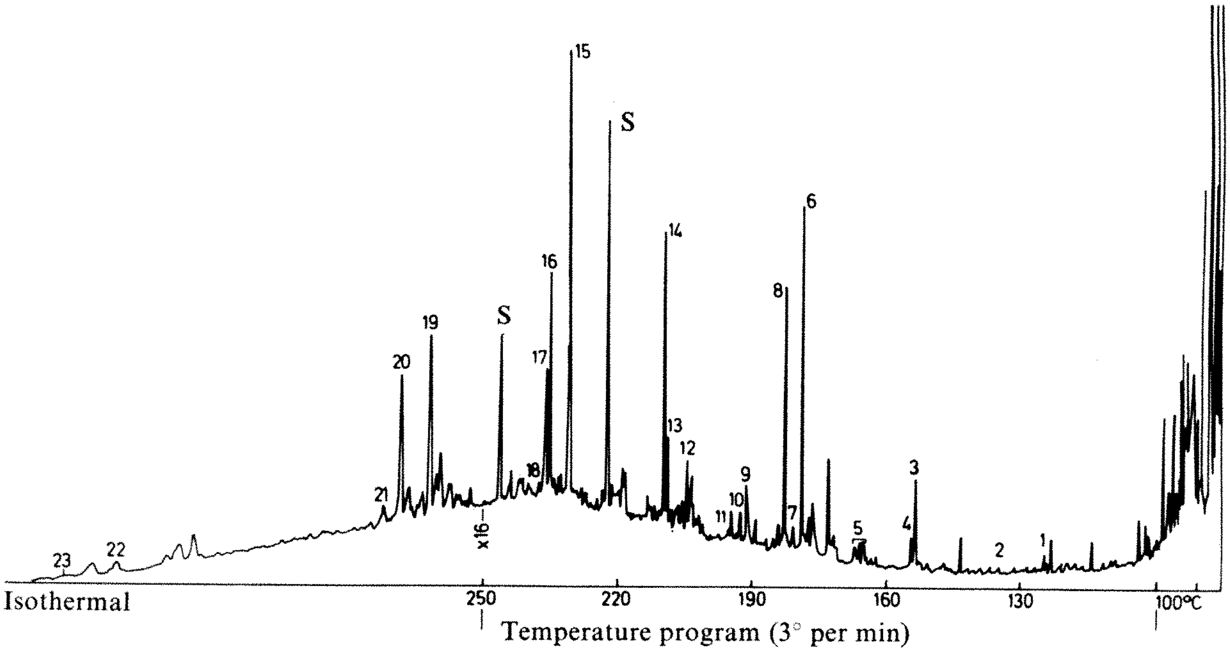

Fig. 1 Gas chromatogram of air filter sample originating from northern England and Scotland. The combined air filters were extracted with 50 ml cyclohexane for 16 h in a Soxhlet apparatus. Before extraction two internal standards, β′β′-binaphtyl and *m*-quaterphenyl, were added. The solution was then extracted with 50 ml dimethylformamide (DMF)/water(9:1). The extraction was repeated with 25 ml of the DMF–water solution. 100 ml water were added to the combined DMF–water solutions and subsequently extracted twice with a total of 75 ml cyclohexane. The cyclohexane-solution was washed twice with distilled water, dried with Na_2SO_4, and concentrated to about 10 ml on a modified Kuderna–Danish apparatus under reduced pressure. The extract was further concentrated to about 0.5 ml on a heatblock (30 °C) under a gentle stream of highly purified nitrogen. The peak numbers refer to the numbers in Table 1. The main components are (3), phenanthrene; (4), anthracene; (6), fluoranthene; (8), pyrene; (15), benzo (*b*&*k*) fluoranthene; (16), benzo(*e*) pyrene; (17) benzo(*a*)pyrene; (18), *o*-phenylenepyrene; and (19), benzo(*ghi*)perylene. S, Internal standards.

Table 1 Concentration of PAH in aerosols (ng per 1,000 m^3)

	Sample number Sampling period	1 20–21 February 1976	2 25–26 November 1975	3 25–27 January 1976	4 1–4 February 1976
		Origin of air:			
	PAH	England, France	Northern England, Scotland	Northern Norway	Stationary air, Southern Norway
3	Phenanthrene	4,725	1,216	36	146
4	Anthracene		278	68	—
5	Methyl-phenanthrene/-anthracene	661	216	—	52
6	Fluoranthene	6,637	3,965	171	324
7	Dihydrobenzo (*a* & *b*) fluorenes	874	363	32	32
8	Pyrene	4,864	3,293	135	286
9	Benzo (*a*) fluorene	815	318	21	26
10	Benzo (*b*) fluorene	571	149	117	148
11	1Methylpyrene	147	99	9	9
12	Benzo (*c*) phenanthrene	1,021	957	38	108
13	Benz (*a*) anthracene	585	740	41	73
14	Chrysene/Triphenylene	1,756	3,269	99	194
15	Benzo (*b* & *k*) fluoranthene	4,312	4,013	83	467
16	Benzo (*e*) pyrene	1,191	2,635	66	135
17	Benzo (*a*) pyrene	965	2,053	59	98
18	Perylene	90	191	Trace	11
19	o-Phenylenepyrene	1,306	1,920	62	144
20	Benzo (*ghi*) perylene	1,142	1,971	64	140
21	Anthanthrene	225	423	7	22
22	Coronene	212	183	—	20
	Total identified PAH	32,099	28,252	1,108	2,435

sarily representative of air that has travelled from England, the concentrations of benzo(*a*)pyrene and some other PAH's are of the same order of magnitude as the mean values reported in recent years in London[7]. The results of this study, though preliminary, show clearly the presence of PAH in atmospheric long-range transported aerosols. Studies have now been extended to incorporate further samples and other sampling stations.

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The microbe thermistor

We describe here the development of a new analytical configuration, called a microbe thermistor, in which immobilised micro-organisms are placed in proximity to a thermistor which measures the metabolic heat evolved by them. Many metabolites can thus be analysed, as we show here by the determination of both single components such as glucose and fructose and of more complex mixtures such as casein. This is in contrast to other analytical applications of immobilised enzymes, for example enzyme electrodes[1–3] and enzyme thermistors[4–6], which can normally allow analysis of only one compound at a time.

We used immobilised whole cells such as *Saccharomyces cerevisiae*, baker's yeast, as a broad specificity sensor. To take full advantage of the generality of such a microbial sensor, a thermistor was chosen as the most suitable transducer, since practically all reactions catalysed by the enzymes of a micro-organism are, in the right conditions, accompanied by temperature changes which can be registered by a thermistor. This is in marked contrast to the situation with bioelectrodes, where the electrode entity can only partly match the 'output' from the sensor because of its specificity for only one or a few different molecular species.

The yeast cells were immobilised in 15% polyacrylamide[7] as follows: Washed baker's yeast cells (5 g wet weight) were suspended in 5 ml of ice-cold water, and mixed with 10 ml ice-cold freshly prepared monomer solution in 0.2 M potassium phosphate buffer, *p*H 7.0, containing 2.85 g acrylamide, 0.15 g *N*,*N*′-methylene-*bis*-acrylamide, 20 µl tetramethylethylenediamine and 10 mg ammonium persulphate. The mixture was immediately poured on to a glass plate provided with stainless steel spacers (0.7 mm), and another glass plate was placed on top of the liquid pool, thus forcing the suspension to form a layer 0.7 mm thick. Polymerisation started within 1 min and was considered complete after 1 h. The gel sheet obtained was passed through a stainless steel mesh giving gel grains of cubic shape, approximately 0.7 × 0.7 × 0.7 mm. Fines were decanted and the yeast-containing grains were finally washed in phosphate buffer for at least 5 h.

To test whether the yeast cells were viable after entrapment in polyacrylamide, the gel grains were disrupted mechanically using a spatula, and the released cells suspended in sterile water, counted under the microscope and spread on malt extract agar plates. Colony counting after incubation for 24 and 72 h at 30 °C revealed that approximately 6% and 8%, respectively, of the released cells had formed colonies. We conclude, therefore, that the entrapment leaves viable cells. It should also be realised, however, that non-viable cells contain several active enzymes[8] and will therefore contribute to the heat formed in an operating microbe thermistor. The reduced viability of the entrapped cells was shown to be due to the toxicity of the monomers (15 min exposure of yeast cells to 15% monomer solution reduced the viability to 3%).

The microbe thermistor was identical to the enzyme thermistor unit described earlier[4]. Approximately 1 g of yeast gel grains were packed in an insulated glass column (0.6 × 4 cm) provided with a thermistor at its outlet. The solution to be analysed was pumped (0.7 ml min^{-1}) through a heat exchanger in an ultra-stable thermostatically controlled water-bath (27 °C) and then through the column. Any heat change occurring in the gel bed alters the resistance of the thermistor and thus gives a signal ultimately registered on a chart recorder (a change in the thermistor temperature of 0.02 °C gave a 100-mV change in the output signal at the highest amplification).

Some basic properties of the enzyme thermistor were deter-

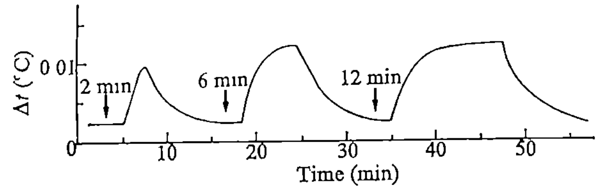
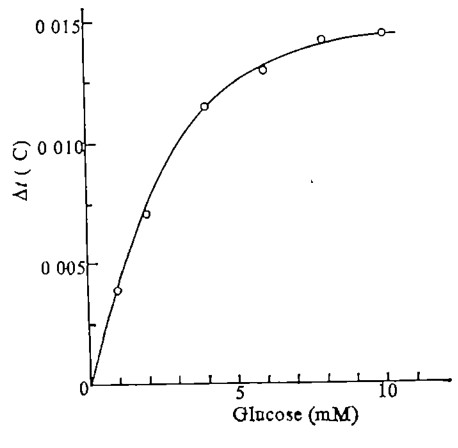


Fig. 1 Experimental curves obtained after injection of 1 mM glucose pulses (in 0.1 M potassium phosphate *p*H 7.0) of varying durations. Arrows indicate substrate introduction.

mined with glucose as substrate. Typical response curves shown in Fig. 1 are qualitatively quite similar to those observed with enzyme thermistors. On increasing the pulse length, an increased heat response was obtained until a constant level of heat production was reached. The correlation between heat production and glucose concentration (Fig. 2) is almost linear at low glucose concentrations, but at 10 mM and higher substrate concentrations a typical saturation behaviour is seen.

The microbe thermistor is thus of value for continuous analysis, but in its present state of development it cannot compete with enzyme electrodes, for example, with respect to speed of analysis. A faster response could probably be achieved by lowering the substrate/product diffusion time through the gel using surface-bound microbes and by increasing the substrate turnover choosing other microorganisms with higher metabolic activity. As expected, the microbe thermistor also proved to be sensitive to other compounds, although the response varied with the compound under study. Fructose (in 0.1 M potassium phosphate, *p*H 7.0) gave a heat signal approximately 60% of that found with an equal concentration of glucose, as well as a slower response and a faster decline back to the baseline. The high molecular weight substrate casein (1% in 0.1 M potassium phosphate buffer, *p*H 7.0) also gave a good response (Δt = 0.014 °C) but with a pronounced lag period before a steady-state level was reached. If, however, the casein was predegraded with Pronase, then a faster response was obtained, but with an unaltered final amplitude (measured after 30 min). The rate in the initial period was about twice as high in this case. Thus the entrapped cells can only degrade high molecular weight compounds with difficulty. The same phenomenon, but even more pronounced, was observed using 1% amylose, which gave no heat signal unless pretreated with amylase (batch- or in line enzyme column-wise). After hydrolysis the response was as fast as shown in Fig. 1. The model system studied here thus allows a differentiation between low and high molecular weight sugars. The preparations of entrapped yeast cells were stable for at least one month at 4 °C and for about 7 d during continuous operating conditions, after which time clogging occurred.

Fig. 2 Measured peak height (Δt °C) as a function of glucose concentration. The glucose substrate was dissolved in 0.1 M potassium phosphate and injected as 1-min pulses.



In another set of experiments, the sensitivity to various inhibitors was analysed (Fig. 3). Thus, on adding 2,4-dinitrophenol (1 mM) to the 1 mM glucose solution, an increased heat response was observed (+20%).This may be interpreted as a result of the uncoupling of the oxidative phosphorylation, preventing conservation of metabolic energy (ATP) which in turn leads to an increased heat production. These results are analogous to the findings reported when dinitrophenol was added to a culture of the microalga *Scenedesmus*, being pumped continuously through a conventional flow microcalorimeter[9,10]. On the other hand, 2 mM arsenate (which is known to influence the glyceraldehyde-3-phosphate dehydrogenase catalysed step) decreased the observed heat production from 1 mM glucose, as seen in Fig. 3.

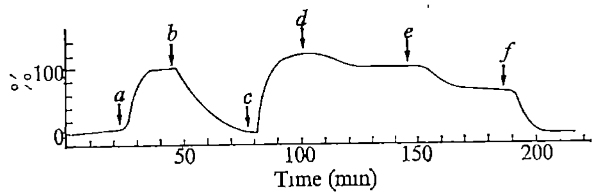


Fig. 3 Thermogram obtained with the microbe thermistor following introduction of various metabolites and inhibitors dissolved in 0.1 M potassium phosphate buffer *p*H 7.0 into the flow. The arrows indicate addition of *a*, 1 mM glucose; *b*, buffer; *c*, 1 mM glucose+1 mM 2,4-dinitrophenol; *d*, 1 mM glucose; *e*, 1 mM glucose+2 mM arsenate; *f*, buffer. The steady-state response to 1 mM glucose is set as 100%.

The use of immobilised microorganisms in combination with suitable transducers offers a flexible alternative of great potential for the analysis of particularly complex solutions. Other organisms or alternative immobilisation techniques could be used in the microbe thermistor. In conclusion, the studies discussed here with the simple microbe thermistor device suggest the following potential areas for its application: (1) its use in analytical determinations of various single components (glucose, fructose) or of more complex solutions (casein); (2) its use in biochemical and microbiological studies, since it conveniently allows successive additions of, for instance, metabolic inhibitors (DNP) or media to the immobilised microbial preparation present in the flow system; and (3) it should also be useful in environmental and process control, as a general indicator for any compound that may affect the metabolism of the microorganism.

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Interspecific competition between great and blue tit

Although competition theory predicts that two species can coexist if intraspecific competition, between members of each species, is more important than interspecific competition, between members of different species, or if $\alpha < K_1/K_2$ and $\beta < K_2/K_1$ (with α and β competition coefficients and K_1 and K_2 carrying capacities for species 1 and 2), there were no animal field studies where these predictions are borne out. This letter, however, explains how two closely related titmice, although competing for food during the breeding season, coexist in broad leaved woods.

The great tit *Parus major* and the blue tit *Parus caeruleus* are two sympatric hole nesting passerines with deciduous forest as their optimal habitat. In winter, they consume different foods but during the breeding season they feed to a large extent on the same[1–4]. Kluyver[5] has, therefore, suggested twice that interspecific competition between the two tits might occur, but he provided no evidence for this. British authors[2–4,6] on the other hand, have explained that this food niche overlap is possible because food would be superabundant during the breeding season and therefore competion would not occur. The data from the Ghent study (Table 1) show that great tit reproduction is significantly reduced both by higher great tit and by higher blue tit numbers. This is the result of a reduction of first brood clutches (strong intra-, weak interspecific effect), of an increase in nest mortality (strong intra-, strong interspecific effect), and of a reduction in the proportion of birds undertaking a second brood (strong intra-, and strong interspecific effect).Thus fewer young are produced per pair and per season (very strong intra- and very strong interspecific effect). Blue tit reproduction is affected in a similar fashion but the effect of the great tit is much less pronounced and is only statistically significant on nest mortality.

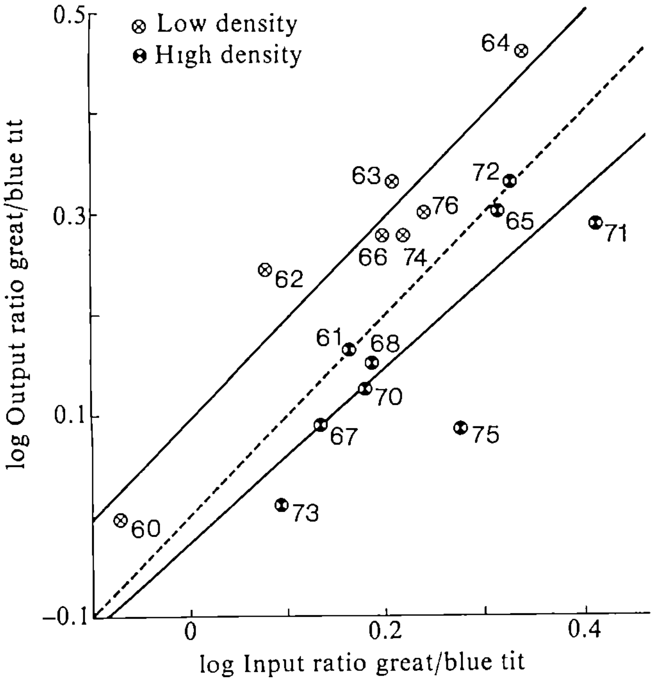

Fig. 1 In plant ecology, Dewit's experimental set up is used to compare the relative competitive ability of two species in different circumstances and at varying relative frequencies, but at the same combined density[7]. If the regression of log input ratio (here log density great tit/density blue tit) against log output ratio (here log number of young fledged great tit/blue tit) lies above the line with slope one through the origin, the species in the numerator does relatively better than the species in the denominator. If the regression line lies below this reference line the reverse is true. As this technique relies on experiments with the same combined density, I selected 7 yr with low combined densities (17–25.8 pairs per 10 hectares, open symbols), and 9 yr with high combined densities (28 1–33 pairs per 10 hectares, partly filled symbols). The low density line lies above, the high density line lies below the line with slope 1 through the origin (stippled), and their slopes are not significantly different from 1 (low density line: slope 1.01, $s_b = 0.14$; high density line slope 0.87, $s_b = 0.22$). This means that the relative frequency does not, but the combined density does affect the relative competitive ability of the tits.

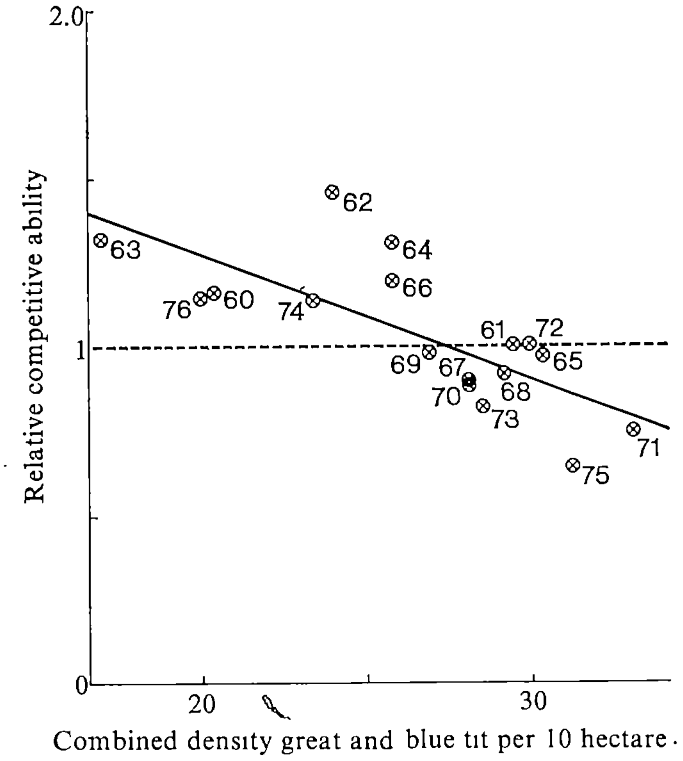

Fig. 2 In Fig. 1, the years were grouped according to low or high combined density but since in this field study densities were not controlled this approach might be criticised. Because combined density seemed to be the factor determining which species will produce relatively more young, the relative competitive ability (ratio of great tit over blue tit density divided by ratio of great tit over blue tit total number of young fledged) is plotted against combined density. The correlation coefficient is highly significant ($r = -0.75$, $F_{1,15} = 19.18$, $P < 0.001$), confirming that the relative competitive ability is dependent on combined density.

All these effects can be related to food. When less food is available per bird, because more birds of either species are present, clutches will be smaller, nest mortality will be higher and it will be more difficult for the females to make up for the weight losses incurred while raising their first brood and thus harder to start a second clutch. Although it could be argued that interference between the birds would have the same result this seems unlikely since (1) there are no observations to support that intra- or interspecific interactions are numerous during the nesting period and especially not when young are being raised (tits being highly territorial species), and (2) the effect of the larger great tit on the smaller blue tit should be more pronounced than vice versa, while in fact the opposite is the case.

Therefore, these negative effects of numbers on reproductive parameters are the result of intra- and interspecific competition for food of the scramble or exploitation type, rather than a possible result of interference competition. This leads to the conclusion that food cannot be superabundantly available during the breeding season.

Why then does the smaller and less numerous blue tit have a stronger effect on the larger and more numerous great tit, than the other way round? To explain this, I hypothesise that the niche of the great tit during the breeding season must be narrower and be contained to a large extent in the broader

Table 1 Intra- and interspecific effect on several reproductive parameters of great and blue tit

Parameter	Density great tit	Density blue tit	Multiple correlated coefficient	Partial correlated coefficient Blue tit constant	Partial correlated coefficient Great tit constant
Great tit					
Clutch size (1st brood)	−0.54*	−0.38	0.61*	−0.51*	−0.33
% second broods	−0.68**	−0.54*	0.80***	−0.70**	−0.57*°
Fertility	−0.55*	−0.48°	0.67*°	−0.53*	−0.45°
Nest success	−0.55*	−0.56*	0.71**	−0.54*	−0.55*
Productivity	−0.69**	−0.64**	0.86**	−0.76***	−0.72***
Blue tit					
Clutch size (1st brood)	−0.42°	−0.54*	0.62*	−0.38	−0.51*
Fertility	−0.27	−0.32	0.38	−0.22	−0.28
Nest success	−0.45°	−0.65**	0.73**	−0.43°	−0.64**
Productivity	−0.39	−0.46°	0.55°	−0.34	−0.43°

The Ghent study was started by J. Hublé in 1959 and is at present supervised by R. Eyckerman. The data used are the weighted averages over the period 1960–1976, from the two best study areas in use since the beginning of the project—Zevergem, an oak wood of 16 hectares (13 hectares until 1963) with 65 nestboxes per 10 hectares, and Maaltepark, a mixed deciduous park of 10 hectares with 78 nestboxes (more details on the study areas in ref. 11). Thus nestboxes were always well in excess. Breeding parameters were determined from weekly controls of all nestboxes throughout the breeding season. Density is the number of breeding pairs per 10 hectares; mean clutch size is calculated from all complete clutches; fertility is the mean number of eggs laid per pair per season, including incomplete, repeat and second clutches; nest success is the proportion of eggs giving rise to fledged young; productivity is the mean number of young fledged from all nests per pair and per season. In our study areas nearly all great and blue tit pairs use nestboxes for breeding (personal observation, personal communication R. Eyckerman)[11].

$^{\circ}P < 0.05$ $^{*}P < 0.025$ $^{*\circ}P < 0.01$ $^{**}P < 0.005$ $^{***}P < 0.001$ (one-tailed test).

feeding niche of the blue tit. The detailed observations in English deciduous forests seem to confirm this hypothesis: Gibb[3] noted that during the breeding season blue tits feed to a large extent on twigs and buds, whereas the heavier great tit uses stouter parts of the tree. These are also used by the blue tit. Betts[4] showed that Lepidoptera larvae, in May, make up the bulk of the food for both species: 84% for the great tit and 56% for the blue tit. 41% of blue tit food (Lepidoptera pupae and Coccidae) is not taken by the great tit (and inaccessible?) whereas only 7% of the great tit diet (adult Coleoptera) is not taken by the blue tit.

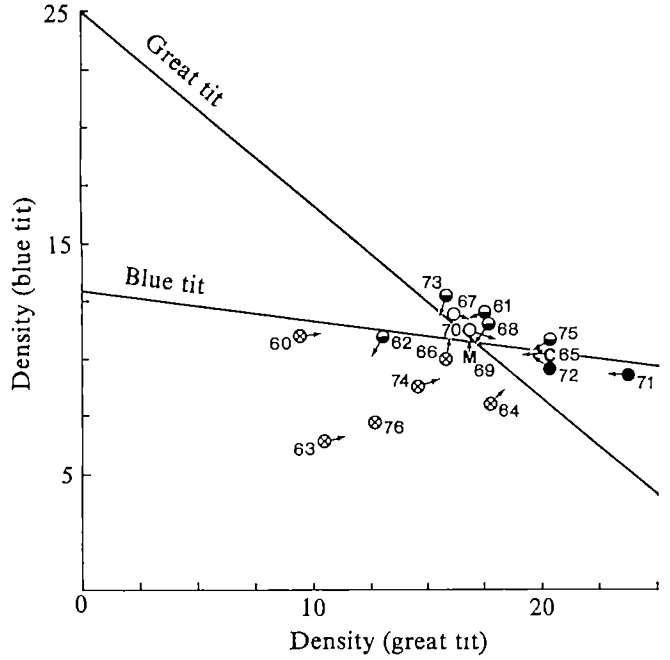

Fig. 3 Equilibrium lines for each species were estimated by requesting eight colleagues to fit the best line for each species so that the points showing an increase until the next year would be below it, the points showing no change on it, and the other points above it. The lines drawn are the averages of these estimates. Although the equilibrium lines varied, in all cases they intersected in such a way that a stable equilibrium would be obtained. The point for 1962 was disregarded because the winter 1962/63 was extremely cold and mortality, especially of the juveniles, was unusually high. The intersections of the equilibrium lines with the axes give: for the great tit $K_1 = 30$, $K_1/\alpha = 25$, and $\alpha = 1.2$; for the blue tit $K_2 = 13$, $K_2/\beta = 100$, and $\beta = 0.13$. ⊗, both species increase; O, both species decrease; ●, blue tit increases, great tit decreases; ◓, great tit increases, blue tit decreases; M: great tit unchanged, blue tit increases; C: blue tit unchanged, great tit decreases.

This way, when it becomes scarce food unaccessible to great tits is still available for blue tits. It is thus shared with fewer competitors and will last longer.

The question that remains is how these two competing titmice coexist in the same preferred habitat. Dewit has shown that two plant species can coexist if the rarer species always has the competitive advantage[7]. Figure 1 shows, using an adapted version of Dewit's method, that the ratio of great versus blue tit numbers does not alter their competitive ability. However, at low combined densities the great tit has the competitive advantage, whereas at high combined densities the blue tit does better, and produces relatively more young. This suggests that the main factor influencing their relative competitive ability would be combined density. This is confirmed in Fig. 2. At a combined density of 27.5 pairs per 10 hectares the relative competitive ability is one and neither species has the advantage. At lower combined densities the great tit produces proportionally more young that the blue tit and at higher combined densities the reverse is true. As long as combined density fluctuates sufficiently both species will coexist. Since great tit numbers vary more than blue tit numbers this will probably result in a stable equilibrium: at low combined densities, great tit numbers will be small and they will increase more than blue tit numbers; this will result in high combined densities and great tits will decrease more than blue tits.

However, it is better to try and estimate competition coefficients and K-values. This is done in Fig. 3 and yields: $\alpha = 1.2$, $K_1 = 30$; $\beta = 0.13$, $K_2 = 13$ (1 = great tit, 2 = blue tit). Thus the effect of the blue tit on the great tit is much larger than vice versa confirming the result obtained in Table 1. The necessary conditions for a stable equilibrium are met since $1.2 < 30/13 = 2.3$, and $0.13 < 13/30 = 0.43$. This means that for both species intraspecific competition is more important than interspecific competition and that, therefore, the great and blue tit can coexist. The stable equilibrium point is at 17 pairs of great tit and 10.5 pairs of blue tit per 10 hectares. Remarkably, this gives a combined density of 27.5 pairs per 10 hectares, which was the stable equilibrium point found in Fig. 2. I suggest that intraspecific competition is mainly for space, as both species are known to be highly territorial and it has been shown that breeding numbers in optimal habitats, for the great tit at least, are limited through territorial behaviour (in spring and in autumn)[8–10], whereas interspecific competition is mainly for food during the breeding season.

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Cercal ablation modifies tethered flight behaviour of cockroach

ANALYSES of insect flight demonstrate a central rhythmic programme modified by means of cephalic and thoracic receptors to allow steering and power output adjustments[1–3]. In orthopteran insects, cercal receptors have never been implicated in flight control, although consideration of their function in cockroaches as directional air current detectors[4] makes this rather surprising. I report here that removal or inactivation of one cercus in a tethered flying cockroach leads to asymmetrical wing movements during flight. Removal of both cerci causes inactivation of forewings and symmetrical beating of both hind wings at a reduced angle to the abdomen. These results suggest cockroach cerci function as specialised equilibrium organs and may relate to the function of cercal interneurones perhaps including the well studied giant fibres (for which there is now no suggested function[5]).

The proterga of unanaesthetised cockroaches (*Periplaneta americana*) were attached by sealing wax to an orthogonal system of three threads which were each further attached to the metal spring of a resistive strain gauge transducer. This allowed limited translation and rotation of the cockroach in three dimensions (Fig. 1). Flight is a complicated behaviour pattern, but for the purposes of this report it is sufficient to describe the approximate angles of the wings with respect to the body and the occurrence or non-occurrence of rhythmic beating. It is unnecessary to present detailed strain gauge measurements. All angles are measured clockwise relative to the median saggital plane of the suspended cockroach seen in frontal view.

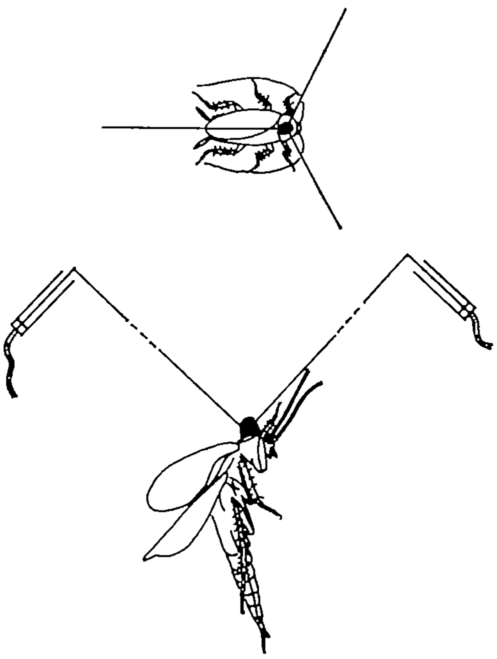

Fig. 1 Dorsal and lateral diagrammatic views of cockroaches suspended by threads from the springs of strain gauge transducers arranged orthogonally. Not to scale.

Suspended cockroaches would fly spontaneously, or could be made to fly by a short puff of air to the head; by making and then breaking tarsal contact with, for example, a finger, or by forced oscillation of the supporting threads. They would often fly for 2 or 3 min. Only one out of 13 cockroaches used did not fly well. After ether anaesthesia in early experiments, cockroaches required 3 d for recovery before they flew well. In normal symmetrical flight (Fig. 2*a*), forewings are held at approximate angles of 90° and 270° for right and left wings respectively and oscillated rhythmically. Hind wings are held at angles of about 120° or 240° and also oscillated. If the threads are asymmetrically fixed to the protergum, so that the cockroach is suspended asymmetrically then the wings on the down-tilted side are held out and oscillated for a longer time than the wings on the other side, and the down-tilted side forewing tends to remain out after all wing oscillation has stopped. If weak flight is initiated, the wings on the uptilted side are not used. If stronger flight is initiated, all four wings are extended and oscillated, but the hind wings on the uptilted side are held closer to the abdomen at angles of 160° or 200°, very near their closed position. As flight continues, the up-tilted side wings tend to be folded away while the down-tilted side wings continue to beat. Compensatory displacements of leg and abdomen are also produced. The effects of tilting are similar to those produced by single cercus ablation (Fig. 2*b*, *c* and *d*).

Removal of one cercus causes similar asymmetrical flight in a previously symmetrical flier. Thus when the right cercus is removed (Fig. 2*b*, *c* and *d*) the wings on the right side continue to be held and oscillated during flight as for normal symmetrical flight in the intact animal. The hind wing on the left side, however, is held and oscillated near its closed position at an angle of 200°, and in time, ceases to be used. The forewing, may be held extended and oscillated at first, but it is soon withdrawn, leaving the hind wing oscillating. The main torque is exerted by the right wings and will tend to turn the cockroach clockwise (seen from the front). Compensatory movements of abdomen and legs consisting of a tilting of the abdomen to the left and extension of left legs and flexion of right legs are usually apparent. These compensatory movements will tend to turn the animal in the same direction as torque from the wings.

Removal of both cerci abolishes normal tethered flight (Fig. 2*e*). Forewings remain folded, but hind wings are held at angles of 160° and 200° and oscillated.

These reflexes were highly stable and easily evoked. When a cockroach which was asymmetrically suspended so that it tended to beat its left wings only, had its right cercus removed, it then beat its right wings in the normal flight manner and ceased to use its wings on the left. It was not necessary to remove the cerci to evoke these reflexes. Interference with the sensory hairs by smearing with mineral jelly caused asymmetrical flight as if the inactivated cercus had been removed. After removal of the jelly, flight was symmetrical as before.

It seems clear that the cerci should be regarded as equilibrium organs which control equilibrium and compensatory reflexes important in flight. This suggested equilibrium function is not incompatible with existing suggested functions for cerci as auditory organs, vibration receptors or air movement detectors[4]. Bischof on electrophysiological evidence has assigned a gravity

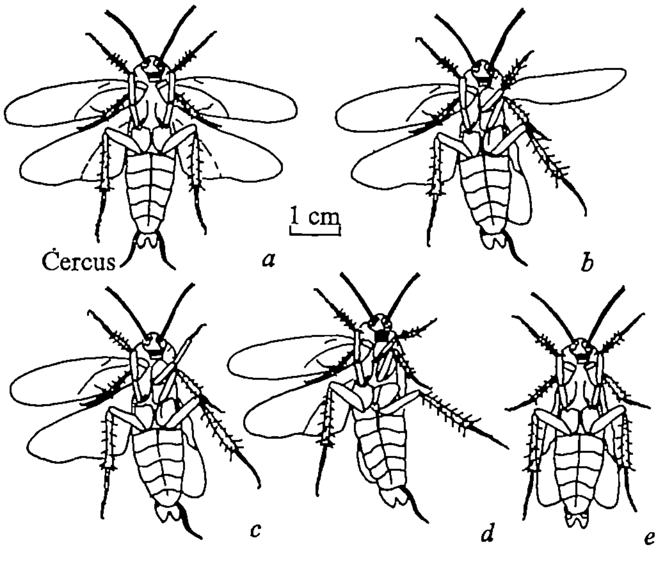

Fig. 2 Frontal views of typical wing and leg positions during tethered flight. *a*, Intact, symmetrically suspended cockroach; *b–d*, right cercus removed showing successive lack of use of left wings; *e*, both cerci removed. Hind wings only are oscillated and are not fully extended.

reception function to club-shaped hairs on the cerci of a cricket[6]. The simplest explanation for the effects reported here is the gravity receptors on cockroach cerci control equilibrium reflexes involving wings and legs in an analogous way to the control of eyestalk and appendage movements by the crustacean statolith[7]. But during inherently unstable locomotion such as flying, an angular movement detector might be expected, analogous to the semicircular canal system of crabs[7,8], facilitating rapid activation of compensatory reflexes. The directional wind sensitive hairs of the cerci could function in this way to detect changes in the velocity of air relative to the cerci which would be caused by angular displacement of the cockroach during forward flight (although many other widely distributed mechanoreceptors, could provide such information[9]).

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Transfer of mitochondria by protoplast fusion in *Saccharomyces cerevisiae*

INTRASPECIFIC and interspecific transfer of nuclear genes of fungi has recently been achieved by fusion of protoplasts[1–6]. We describe here the transfer of mitochondrial genome using the protoplast fusion technique. The experiments were carried out with strains of identical mating-type, so we also report successful complementation of auxotrophic yeast cells of the same mating-type after protoplast fusion.

The main steps of the experiments were as follows: (1) selection of a mitochondrial donor with stable nuclear and mitochondrial markers, and of a recipient with a nuclear colour marker and ρ^0 petite mitochondrial character. In both cases it was an additional requirement to have strains of identical mating-type to exclude the possibility of mitochondrial transfer by zygote formation; further, rapid and complete protoplast formation, followed by high-frequency reversion to intact cells, was also a basic necessity. (2) Protoplast formation. (3) Induction of fusion of donor and recipient protoplasts and selective regeneration of those undergoing complementation. (4) Characterisation of the fusion products. And (5), recovery of the recipient containing the marked mitochondria of the donor.

The uracil and tryptophan-requiring strain IL 216-1B (nuclear genotype: α *ura*1, *trp*1; mitochondrial genotype: ω^+ E^R_{221}) was selected as mitochondrial donor. The oxygen-consumption of log-phase cells in yeast extract–glucose medium was 21.8 μl O_2 per mg wet weight per h. This strain can grow on non-fermentable carbon sources in the presence of a high concentration (5 mg ml^{-1}) of erythromycin. A ρ^0 petite mutant of the erythromycin-sensitive (0.2 mg ml^{-1}) adenine-requiring grande strain DV 147 (nuclear genotype: α, *ade*2) was selected as mitochondrial recipient. In the petite mutant no mitochondrial DNA was detected by sensitive fluorescent staining techniques using 4′,6-diamidino-2-phenylindole (DAPI) (refs 6, 7) or 2-[2-(4-hydroxyphenyl)-6-benzimidazolyl]-6-(1-methyl-4-piperazyl) benzimidazole.3HCl (33258 Hoechst)[7] in CsCl gradients. The recipient showed no respiration and was unable to grow on non-fermentable carbon sources or galactose. On minimal medium supplemented with adenine (optimum 5 μg ml^{-1}), the grande DV 147 strain formed fast-growing red colonies, while the colonies of the petite mutant were slow-growing and white.

For protoplast formation the cells were cultivated in yeast extract–glucose medium, collected in the early log-phase, treated with 1% 2-mercaptoethanol[8] for 10 min, collected by centrifugation, resuspended in 0.7 M KCl containing 0.1% 2-mercaptoethanol and 1% snail enzyme to give a final cell concentration of 10^8 ml^{-1}, and gently shaken at 30 °C. All the cells were converted into protoplasts in 1 h; these were collected and washed twice with 0.4 M $CaCl_2$ by centrifugation, and the two strains were then mixed in a 1:1 ratio (5×10^7 protoplasts each). The fusion process was carried out with 35% polyethylene glycol (PEG, molecular weight 4,000) as described previously[2,9]. The suspension of aggregated protoplasts was diluted with 35% PEG, mixed at 43 °C with vitamin-containing[10] minimal medium supplemented with 0.4 M $CaCl_2$ and 2% agar, and poured as a thin top layer on to a similar medium. In this medium, only fusion products complemented nutritionally by heterokaryon or diploid (polyploid) formation between the two partners taking part in the fusion process can regenerate and grow into colonies of wild phenotype.

No colonies appeared if intact cells or protoplasts of only one of the partners were applied; if intact cells of the two partners were cultivated together for mating; or when no PEG treatment was used for aggregation of protoplasts. This means that no back-mutation was observed; no complementation occurred with intact cells of identical mating type; and PEG treatment is essential to induce the fusion process of yeast protoplasts. On the other hand, good regeneration and colony growth was observed in all these cases in nutritionally-sufficient agar media.

The complementation frequency proved to be about 0.04% if all protoplasts taking part in the fusion process were considered, and about 0.6% if the number of colonies growing in minimal medium was compared with the number developing in nutritionally-rich medium. In the present fusion system, the frequencies obtained were similar to those not only for other α+α or *a*+*a*, but also for α+*a* pairs of protoplasts (unpublished), indicating that the protoplast fusion is not affected by mating factors.

The main characteristics of the nutritionally-complemented and regenerated fusion products were as follows. On or in minimal and rich media their cultures, always white, grow more intensively than the fusion partners in optimum conditions, with increased formazan production if tetrazolium violet (20 μg ml^{-1}) was applied in the media. These properties are indications of enhanced metabolic processes. In the fusion products only one nucleus per cell was demonstrated by staining with acridine orange[11]. The average cell size of the fusion products was significantly larger than that of either of the partners. The sizes and DNA determination data are given in Table 1. DNA was extracted by the Bostock procedure[12] and estimated with diphenylamine[13,14] using chicken erythrocyte DNA as standard. A bracketed symbol [x+y] is proposed for the fusion products, at least until they have been characterised thoroughly.

Table 1 Cell sizes and DNA contents of the fusion partners, fusion products, and erythromycin-resistant haploid and aneuploid segregants.

Strains	Length (μm)	Width (μm)	DNA content (fg DNA per cell)
IL 216-1B ρ^+ E^R	5.37±0.14	4.82±0.12	24.4±1.8
DV 147 ρ^0	4.73±0.16	4.43±0.13	22.5±2.3
[IL 216-1B ρ^+ E^R+DV 147 ρ^0]	7.57±0.15	5.42±0.15	48.0±4.1
DV 147 ρ^+ E^R (haploid)	5.62±0.17	5.30±0.16	22.5±0.3
DV 147 ρ^+ E^R (aneuploid)	5.55±0.14	5.04±0.13	30.7±0.6

Values are ± s.e.m. Standard errors are based on 50 measurements and at least three DNA determinations.

These data, together with the observation that the fusion products can be induced to undergo haploidisation, suggest that the fusion products were diploids. No spore formation and only rare spontaneous haploidisation, with a frequency of less than 10^{-4}, has yet been demonstrated. All fusion isolates were resistant to erythromycin at the same level as the donor strain.

By induced haploidisation with *p*-fluorophenylalanine[15-16] (400 to 600 μg ml^{-1}) or with benomyl[17-18] (6–15 μg ml^{-1}) in yeast extract–glucose medium, up to 40% adenine-requiring haploid and aneuploid red colonies with α genome could be isolated. These colonies consisted of respiration-sufficient cells harbouring mitochondrial resistance to erythromycin, indicating successful mitochondrial transfer. Among the resulting adenine-requiring E^R isolates, several proved different from the original DV 147 strain in certain properties. One was the generation of a much lower amount (< 2%) of spontaneous petites, in contrast to the case with the original strain (up to 60%). These properties are under investigation.

Mitochondrial transfer by protoplast fusion—'transfusion' of mitochondria—may contribute considerably to mitochondrial biochemistry and genetics. Not only intraspecific, but also interspecific mitochondrial transfer and recombination of mitochondrial DNA of distant species seems feasible and this possibility is currently under study. It additionally seems of interest to exploit the possibility of fusion of protoplasts of identical mating-type and in this way to build up various α, *a* or specially mixed nuclear genomes for basic research and practical applications, by overcoming the mating-type barrier.

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Circular linkage map of *Rhizobium meliloti* chromosome

GENETIC studies on symbiotic nitrogen-fixing rhizobia are greatly hampered by the lack of an efficient system for genetic mapping in rhizobia. Although there have been reports on transformation[1-3] and transduction[4] in nodulating rhizobia as well as conjugation[5] in a non-nodulating strain of *Rhizobium lupini*, the genetic map of a nodulating and nitrogen-fixing *Rhizobium* strain has not been established yet. Plasmids of the P-1 incompatibility group have a remarkably wide host range and can be transferred to many *Rhizobium* species[6,7]. Some of these plasmids can promote chromosome transfer in *Pseudomonas aeruginosa* strain PAT but show little chromosome donor ability (Cda) in strain PAO[8,9]. Recently a variant (R68.45) of the P group plasmid R68, with high Cda property in strain PAO, was isolated by Haas and Holloway[10]. Plasmid R68.45, unlike most of the sex factors, promotes chromosome transfer for a range of chromosomal genes in *Rhodopseudomonas*, *P. putida*, *Escherichia coli* (B. W. Holloway, personal communication) and in *Rhizobium leguminosarum*[11]. We have found that this plasmid can promote chromosome transfer also in *Rhizobium meliloti* and can be used for mapping of the chromosome. We present here a circular genetic map of the *R. meliloti* 41 chromosome and describe a new approach in the evaluation of conjugational mapping data which may be applicable to other Gram-negative bacteria.

Plasmid R68.45 was introduced into *R. meliloti* 41 strains AK208 and AK209, and the chromosome donor ability (Cda) of the product was investigated. Various auxotrophic and antibiotic resistance markers were used as chromosomal markers in plate matings on nutrient agar[12]. As seen in Table 1, R68.45 could be transferred between *R. meliloti* strains at frequencies of (3–7)×10^{-1} per donor cell and recombinants for various markers were produced at frequencies of 10^{-3} to 10^{-5} per donor cell. This frequency of recombination is about the same as that found in *P. aeruginosa* PAO[10] and about 2–3 orders of magnitude higher than in *R. leguminosarum*[11]. Recombination

Table 1 Plasmid transfer and chromosome mobilisation by R68.45 in *R. meliloti*

Donor	Recipient	Frequency of Tc^R transconjugants	Selected chromosomal marker	Recombination frequency
AK208	AK80	6.3×10^{-1}	*cys*46$^+$	3.6×10^{-4}
			*str*1	1.9×10^{-4}
AK208	AK193	5.2×10^{-1}	*gly*1$^+$	2.8×10^{-4}
			*met*2$^+$	2.6×10^{-4}
AK209	AK80	3.2×10^{-1}	*cys*46$^+$	1.2×10^{-4}
			*str*1	8.0×10^{-5}
AK209	AK193	4.4×10^{-1}	*gly*1$^+$	1.3×10^{-4}
			*met*2$^+$	1.5×10^{-4}

Mating conditions in all experiments were as described before[12]. Selective medium: minimal medium (GTPS[12]) was supplemented with amino acids, 50 μg ml^{-1}; bases, 25 μg ml^{-1}; streptomycin, 100 μg ml^{-1}; rifampicin, 100 μg ml^{-1}; tetracycline-HCl, 15 μg ml^{-1}, as required. Agar surface matings were carried out for 16 h at 37 °C. Bacteria were scraped off the surface of the plates, resuspended in saline and plated on selective media. Counterselection for AK208 and AK209 was by *his*1 and *ura*29, respectively. Strains are described in Table 2. Transfer frequency: Tc^R transconjugants per donor bacterium. Recombinants on same basis.

Table 2 Linkage analysis of various marker pairs of *R. meliloti*

Donor	Recipient	Marker pairs		Linkage c	Distance d	Additive distance
AK213	AK80	*cys*46$^+$	*rif*1	0.10	0.54	
		*str*1	*rif*1	0.73	0.10	
		*cys*46$^+$	*str*1	0.04	0.66	0.64
AK213	AK193	*cys*46$^+$	*rif*1	0.16	0.46	
		*met*2$^+$	*rif*1	0.05	0.63	
		*gly*1$^+$	*rif*1	<0.01	—	
AK208	AK193	*cys*46$^+$	*met*2$^+$	<0.01	—	
		*gly*1$^+$	*ade*4$^+$	0.75	0.09	
		*ade*4$^+$	*met*2$^+$	0.33	0.31	
		*gly*1$^+$	*met*2$^+$	0.22	0.40	0.40
AK208	AK194	*tyr*1$^+$	*gly*1$^+$	0.79	0.08	
		*gly*1$^+$	*ade*4$^+$	0.77	0.08	
		*tyr*1$^+$	*ade*4$^+$	0.66	0.13	0.16
AK208	AK184	*gly*1$^+$	*leu*4$^+$	0.06	0.61	

Strains: AK80: *cys*46 (F46 of ref. 14); by successive mutagenesis with nitrosoguanidine (NTG)—AK193: *cys*46, *gly*1, *ade*4, *met*2, *str*1; AK194: *cys*46, *gly*1, *tyr*1, *str*1; AK184: *cys*46, *gly*1, *leu*4, *str*1. AK213: *his*1, *str*1, *rif*1 (R68.45) by transconjugation of a spontaneous *rif*1 mutant of AK74, *his*1, *str*1 (A1 of ref. 13)—AK208: *his*1, *str*1 (R68.45) by transconjugation of AK74. Counterselection for donors by *his*1; selected for single markers, other marker by replica plating, averaged value for both directions. Strains used in other Tables: AK171 and AK173: *met*33 and *ura*29 mutants by NTG from ZS1, an *ade*1, *str*1 mutant of Z. Sváb; AK209: *ade*1, *ura*29, *str*1 (R68.45) by transconjugation of AK173; GY23: *cys*46, *gly*1, *leu*4, *str*1 (R68.45) by transconjugation of AK184; AK189: *cys*46, *gly*1, *ade*4, *str*1 (R68.45) by transconjugation of plasmid into a two-step NTG mutant of AK80.

frequencies for various markers were essentially the same when different donors were used, so this suggests that R68.45 promotes gene transfer from several chromosomal sites in *R. meliloti*.

We found that R68.45 can lose its Cda property during growth but other properties of the plasmid, such as resistance and transfer markers, are stably inherited. Therefore special care has to be taken to maintain effective donor cultures, as suggested by Haas and Holloway[10]. Recombinants selected for various chromosomal markers were stable but in many instances (10–80%) plasmid markers (TcR or Cda) were partially or completely lost in the recombinants, irrespective of the chromosomal marker selected. These observations also indicate that plasmid R68.45 behaves in *R. meliloti* 41 as it does in *P. aeruginosa* PAO. In recombinants selected for given chromosomal marker and possessing intact R68.45, R68.45 promoted the transfer of the given marker and of various other markers at the same frequency. This suggests that R68.45 is not integrated into the chromosome of the recombinants. Furthermore, using mutants of *E. coli* we demonstrated that the appearance of recombinants after chromosome mobilisation by R68.45 requires *rec*A function, as was expected.

Double or multiply-marked derivatives of *R. meliloti* 41 were mated with derivatives of *R. meliloti* carrying R68.45. Recombinants for single markers were selected and coinheritance of unselected markers was determined by replica plating. The linkage data for certain marker pairs are shown in Tables 2 and 3. In repeated experiments the linkage frequency (number of colonies with the phenotype of both selected and unselected markers per colony showing the phenotype of the selected marker) for a given marker pair varied by up to 10%.

Taking linkage (or cotransfer) frequency to be inversely related to the distance between genes, one usually obtains enough information to establish the marker order, but values of cotransfer frequencies are not additive. To establish a genetic map with additive map distances we attempted to transform our linkage data into additive map distances. We found that the map function derived by Wu[15] for transduction was satisfactory for relating our linkage frequency values (c) to the map distance (d) (in arbitrary units) between two markers

$$c = (1-d)^3$$

We calculated and compared the d values and their additivity, for given marker pairs, obtained in separate experiments and in three-factor crosses. Examples for additive distance values, calculated in three-factor crosses, are shown in Tables 2 and 3. Experimentally determined d values are summarised in Fig. 1. Within the limits of our present experimental data, the correlation is fairly satisfactory for c values higher than 0.1.

There is not enough information about the mechanism of chromosome transfer by R68.45 to justify the use of Wu's equation, derived from the assumption that in transduction the donor fragments have nearly constant length and various regions of this fragment are incorporated into the chromosome of the recipient by pairs of crossover events. Nevertheless,

Table 3 Linkage analysis of the *cys*46 region in *R. meliloti*

Donor	Recipient	Counterselected/ selected markers	Unselected classes of recombinants (%)*			Linkage frequency of pairs, c			Map distance d	Additive distance
GY23	AK171	*gly*1/*ade*1$^+$	*cys*46	*met*33	51	*ade*1$^+$	*cys*46	0.62	0.15	
			*cys*46$^+$	*met*33	38	*cys*46	*met*33$^+$	0.18	0.44	
			*cys*46	*met*33$^+$	11	*ade*1$^+$	*met*33$^+$			0.59
		*gly*1/*met*33$^+$	*cys*46	*ade*1	8	*met*33$^+$	*cys*46	0.14	0.48	
			*cys*46	*ade*1$^+$	6	*met*33$^+$	*ade*1$^+$	0.06	0.61	
			*cys*46$^+$	*ade*1	85	*cys*46	*ade*1$^+$			0.13
		*cys*46/*met*33$^+$	*leu*4	*gly*1$^+$	28	*met*33$^+$	*leu*4	0.28	0.35	
			*leu*4$^+$	*gly*1$^+$	72	*met*33$^+$	*gly*1	<0.01	—	
AK189	AK173	*gly*1/*ade*1$^+$	*cys*46	*ura*29	50	*ade*1$^+$	*cys*46	0.58	0.17	
			*cys*46	*ura*29$^+$	8	*ade*1$^+$	*ura*29$^+$	0.08	0.57	
			*cys*46$^+$	*ura*29	42	*cys*46	*ura*29$^+$			0.40
		*gly*1/*ura*29$^+$	*cys*46	*ade*1	11	*ura*29$^+$	*cys*46	0.20	0.41	
			*cys*46	*ade*1$^+$	9	*ura*29$^+$	*ade*1	0.09	0.55	
			*cys*46$^+$	*ade*1	80	*ade*1$^+$	*cys*46			0.14

Strains and markers: see Table 2.

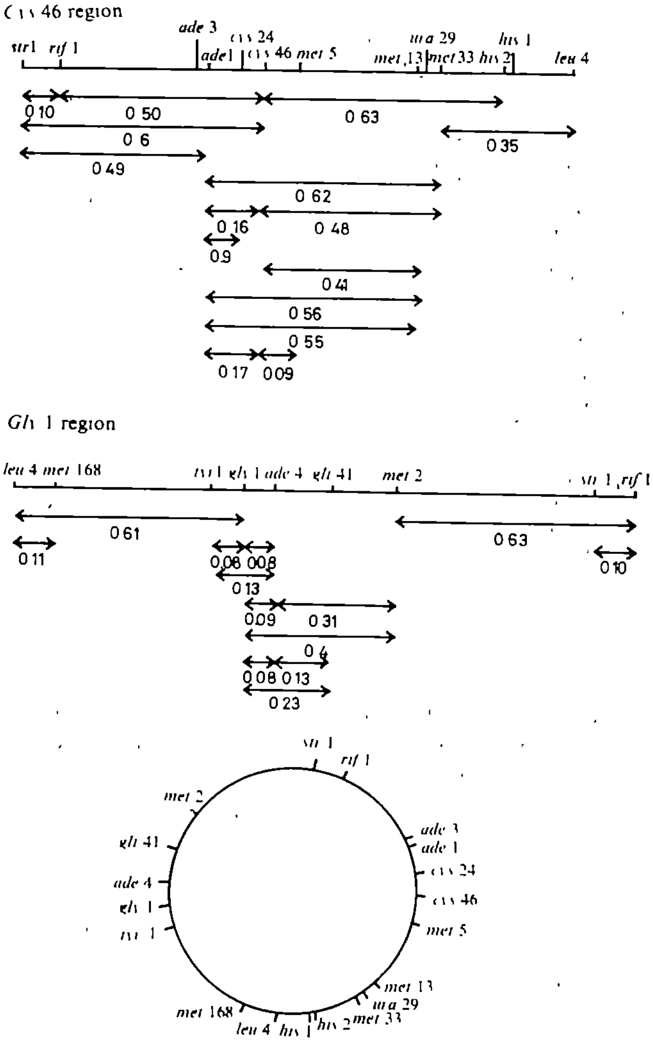
*Unselected marker classes not given were less than 1%.

our data do indicate that the equation is applicable for converting linkage frequencies into approximatively additive map distance values.

The order of markers was determined by calculating map distances between closely linked markers or by three-factor crosses. Data in Table 2 indicate the order *tyr*1–*gyl*1–*ade*4–*met*2–*str*1–*rif*1–*cys*46. Examining the linkage of unselected markers in three-factor crosses gave the linkage data shown in Table 3. From all of these results the order *str*1–*ade*1–*cys*46–*met*33–*ura*29–*leu*4–*gly*1 could be deduced. The results also placed the additional markers *cys*24, *met*5, *met*13, *his*1, *his*2, *met*168 and *glt*41 in relation to the others. By confirming the linking of both ends of the above sequences, a circular map could be established (Fig. 1). The total length of the chromosome is about 3.1–3.2 arbitrary units (*d*). Mutant strains with single markers are being mapped in our laboratory. Using our multiply-marked mutants five matings are enough to locate any mutation on the linkage map.

In the original map function derived by Wu, $c = (1-d/l)^3$ the average size of the transducing particle (l) was known from independent measurements. Applying this equation for our system and using the information that the length of the chromosome is 3.2 d units, the average size of the donor fragment could be calculated. This is about 1/3 of the chromosome. On the other hand, however, in several cases we could demonstrate 0.05–1.0% co-inheritance of markers up to 1.5 d units apart (data not shown). This suggests that about 1% of the donor fragments are at least halves of the total chromosome in size.

Fig. 1 Linkage map of *R. meliloti* 41. Distances between markers are *d* values, obtained directly from independent crosses. Locations of *cys*46, *met*5, *met*13, *his*1, *his*2, *met*168 and *glt*41 were determined in three-factor crosses.



Therefore we could not calculate *d* values from linkage frequency values (*c*), lower than 0.03.

In conclusion, our data indicate that R68.45 is suitable for genetic mapping in *Rhizobium meliloti*. The mapping was based on linkage analysis of chromosomal markers and a circular map of the *R. meliloti* 41 chromosome was constructed. Recently Meade and Signer demonstrated linkage of chromosomal markers of *R. meliloti* 2011, using RP4 as a sex factor[16]. Using R68.45 we are directing our efforts to map those genes which are involved in symbiotic nitrogen fixation.

Although we do not have independent evidence to support our empirical use of the described map function relating linkage frequency to the map distance, we suggest that the map distances between markers are approximately proportional to the physical marker distances around the chromosome. Preliminary experiments in our laboratory indicate that this equation is applicable for linkage frequencies, obtained with R68.45, in *E. coli* as well. Furthermore, this plasmid would be useful for genetic mapping in many other Gram-negative bacteria, and our new approach for evaluating conjugational mapping data will certainly be useful for these future genetic systems.

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Note added in proof: Drs J. E. Beringer and A. W. Johnston have informed us that they have established a circular linkage map of *R. leguminosarum* chromosome[17] and have demonstrated interspecific recombination mediated by R68.45 among various *Rhizobium* species[18]. A linkage map of *R. meliloti* has recently been reported by Meade and Signer[19].

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Very close linkage between HLA-B and Bf inferred from allelic association

The human leukocyte antigen loci (HLA, previously HL-A) are known to be closely linked to the locus defining factor B of the alternative complement pathway[1]. The B-factor locus was previously termed GBG (glycine-rich β-glycoprotein) and is now known as Bf. If the consequences of recombination do not impair survival we may use data on allelic association (sometimes termed linkage disequilibrium) as a rough estimate of the upper limit of the recombination fraction, θ. This approach has advantages

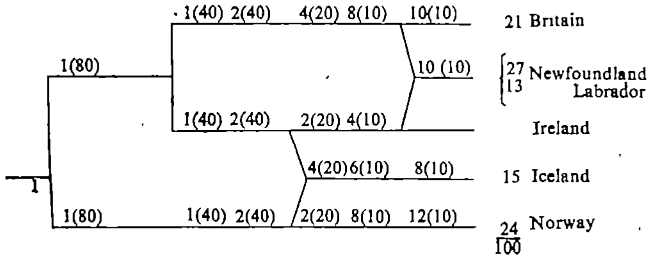

Fig. 1 A 'family tree' of the populations studied. Dimensions are time in generations (in brackets) and assumed numbers of ancestral B8 loci (minimum plausible estimates).

where linkage is very close and the frequency of laboratory and other errors in ascertaining recombinants is greater than the recombination frequency. By typing two or more members of families we have defined 100 apparently independent haplotypes with the HLA-B allele B8 and all had the Bf allele S. These observations were fairly evenly divided between Iceland, north-west Newfoundland and Labrador, Central England and Norway. Figure 1 shows a 'family tree' of these populations.

Iceland was settled in AD 874 by Norsemen with Irish slaves and had a population of over 70,000 by AD 1100; the Nordic and Celtic genetic contributions seem to have been comparable[2]. Newfoundland was settled more slowly, but was substantially settled over 200 yr ago, mainly from England and Ireland. There are few data on the time of separation of the main Celtic, Saxon and Norse groups, or on the scale of the Saxon invasions.

The dimensions of the tree are time in generations (in brackets) and inferred minimal numbers of ancestral haplotypes. The number of individuals bearing the haplotype who were the ancestors of the tested population cannot be known, but can never have been greater than in the tested population. At some point in history all the tested populations had common ancestors and, in the remote past, there must have been a common ancestor of all the tested individuals. We feel that the dimensions of the tree are not unreasonable and are intentionally highly conservative, as is the assumption of a single ancestral haplotype at 3000 BC.

There are no apparent recombinants between the B and Bf loci in our sample. The chance of none of c haplotypes recombining in n generations is $(1-\theta)^{cn}$. The chance of an S allele being replaced by another is the relative proportion of these others, which is about a third in our population, so that the chance of no evident recombinant is $(1-\theta/3)^{cn}$ According to our assumptions, cn over all links is about 2,000. The table shows the probability of B8 alleles being exclusively associated with S on assumptions of various recombination fractions, assuming no selection against recombinants. While mild selection against recombinants will explain a substantial deficiency of B8-F haplotypes, a very strong selective disadvantage would have to be postulated to explain the complete absence of contemporary recombinants in such extensive data.

Table 1 Probabilities of no detectable recombinant in 1,500 and 3,000 meioses

θ	1,500	3,000
0.001	0.60	0.37
0.002	0.37	0.14
0.003	0.22	0.05
0.004	0.14	0.02
0.005	0.08	0.01
0.006	0.05	
0.008	0.02	
0.010	0.01	

We are aware of slight selective bias in selecting the B8 allele for special consideration; however, this allele is of particular interest for other reasons. One B8-F_1 haplotype has been reported by Hauptmann *et al.*[3]. This variant is usually associated with B18 in our experience. Albert *et al.*[4] also found a complete and significant deficiency of B8-F (0/30) haplotypes in Germany, where Bender *et al.*[5] found 4 B8-F haplotypes, 1 B8-S_1 and 64 B8-S, and also reported a B18-F_1 haplotype. B18 is also allelically associated with deficiency of the second component of complement (C2).

We consider our data to be evidence of very close linkage between the loci of B and Bf. If the determinants relating to graft survival are stronger at the B locus or are determined by loci which are closer to this locus than to the A locus, Bf-typing might be of more practical value than HLA-A typing. It may also provide *a priori* evidence suggestive of a serologically definable split when one B allele is commonly associated with more than one Bf allele.

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Factors from 3T3 cells stimulate proliferation of cultured vascular endothelial cells

TUMOURS induce vascularisation into the neoplasm to provide needed cell nutrients and remove wastes[1]. The formation of tumour blood vessels seems to depend, in part, on the proliferation and movement of vascular endothelial cells which are, in turn, controlled by the secretion of tumour angiogenesis factor (TAF) (refs 2–4). Extracts of tumours[2-4] and of established cell lines[5] have TAF activity *in vivo* But little is known about the molecular nature or mechanism of action of TAF. The proliferation, formation and integrity of vascular endothelium are also involved in normal host processes· wound healing, morphogenetic tissue rearrangements, as well as the prevention of various non-neoplastic diseases resulting from blood vessel wall injury depend on angiogenesis factors[6]. A convenient method to study angiogenesis and endothelial repair is with an *in vitro* endothelial cell system, but the lack of uniform, reliable cultured endothelial cell lines has delayed progress in this area. Most studies[7-9] (with some exceptions[10]) have used uncloned vascular cells which are often slow growing and heterogeneous in cell type. We have recently established a cloned adult bovine aortic endothelial (ABAE) cell line which has maintained its endothelial properties for over 1 yr in culture, provided that fibroblast growth factor (FGF) is present in the growth medium[11]. We report here the mitogenic effects of several untransformed and transformed cell lines on cloned ABAE cells and non-vascular endothelial cell controls.

Endothelial cell mitogenesis was monitored by seeding ABAE or control non-vascular adult bovine corneal endothelial (ABCE) cells on to γ-irradiated feeder layers of test cell cultures and measuring the proliferation of the unirradiated endothelial cells in the absence of FGF. All cells were cultured in Dulbecco modified Eagle's medium (DMEM) (ref. 12) with 10% calf serum. When vascular ABAE cells were seeded on to γ-irradiated feeder layers of MSV-transformed BALB/c 3T3 (MSV3T3) or BALB/c 3T3 (clone A·31) cells, substantial proliferation of ABAE cells occurred within two days (Fig. 1*a*). By 12 days ABAE cells grew to almost 100 times the density of ABAE cells grown in DMEM with 10% calf serum (Figs 1*a* and 2). MSV3T3 cells were almost as effective as FGF (100 ng ml^{-1}) and more effective than BALB/c 3T3 cells in promoting growth of ABAE cells, while NIH Swiss 3T3 and ABAE feeder cells had little effect on ABAE cell mitogenesis (Fig. 1*a*). When non-vascular ABCE cells were substituted for ABAE cells, none of the cell lines tested stimulated ABCE cell proliferation, although ABCE cells do respond to FGF (Figs 1*b* and 2). Several other γ-irradiated untransformed cell lines were tested for their ability to promote the growth of ABAE cells. Although some of these cell lines caused slight increases in ABAE cell growth (15–41% over controls), none of the lines was judged to be significantly mitogenic to ABAE cells (Table 1). Thus far, every transformed cell line tested has stimulated ABAE but not ABCE cell proliferation (C.R.B. and G.L.N., to be published).

The proliferative effects of γ-irradiated BALB/c 3T3 and MSV3T3 cells on ABAE cells were due to substances released by the feeder cells and were not due to artefacts of γ-irradiation. This was demonstrated by experiments where the effects of conditioned medium from unirradiated feeder cells on ABAE cell proliferation were measured. Conditioned medium from BALB/c 3T3 and MSV3T3 cells stimulated growth of ABAE cells approximately 20 and 30 times, respectively, over ABAE cell growth in DMEM with 10% calf serum (Fig. 3). Although this was less than the effect of γ-irradiated BALB/c 3T3 or MSV3T3 cells on ABAE cell proliferation, it indicates that the former cell lines release an ABAE growth promoting factor(s) into the medium. Conditioned medium from ABAE cells was slightly more effective than γ-irradiated ABAE cells in promoting proliferation of the same cells but was much less effective than conditioned medium from BALB/c 3T3 or MSV3T3 cells. Conditioned medium from unirradiated BALB/c 3T3 or MSV3T3 did not stimulate proliferation of non-vascular ABCE cells (data not shown). These effects were not due to γ irradiation, because conditioned medium from γ-irradiated cells had the same effect as conditioned medium from non-irradiated cells, and mitomycin c-treated cells produced similar prolifera-

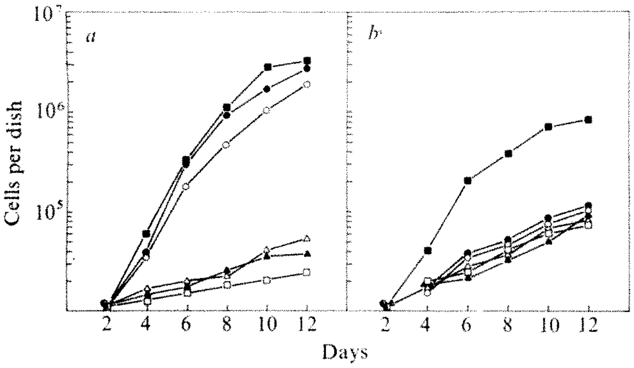
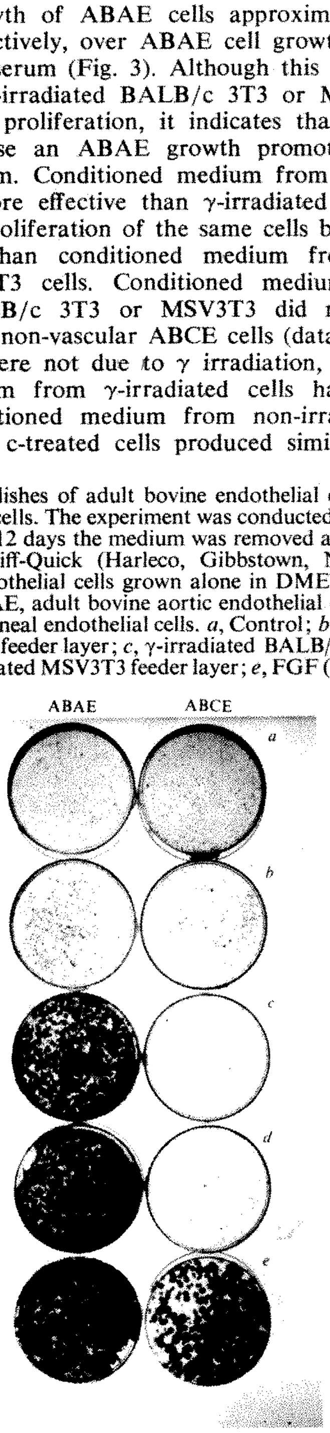


Fig. 1 Growth of adult bovine endothelial cells on γ-irradiated cells. *a*, Aortic endothelial cells; *b*, corneal endothelial cells. The cloning, culturing and characterisation of adult bovine aortic[11] and corneal[13] endothelial cells have been previously described. The aortic endothelial cells are routinely cultured in the presence of 100 ng ml^{-1} of FGF, which was purified as described[14,20]. BALB/c 3T3 cells (clone A·31) and MSV3T3 cells (murine sarcoma virus-transformed clone A·31 BALB/c 3T3 cells) were obtained from Dr S. Aaronson of the National Cancer Institute, Bethesda, MD. BALB/c 3T3 cells grew as a density-inhibited line and were non-tumorigenic when 10^6 cells were injected subcutaneously into BALB/c mice, whereas MSV3T3 cells had lost density-inhibition of growth and formed large tumours when 10^6 cells were injected subcutaneously into BALB/c mice. NIH Swiss 3T3 cells were obtained from Dr K. Jones of the University of California, San Diego. All cell lines were cultured at 37 °C on Falcon tissue culture dishes in Dulbecco modified Eagle's medium (DMEM)[12] supplemented with 10% calf serum (Gibco). The *in vitro* assay of endothelial cell proliferation using γ-irradiated cells was conducted as follows. Six dishes were seeded 10,000 test cells in DMEM with 10% calf serum, and after the cells had attached (4–12 h), the dishes were γ-irradiated with a total of 6,000 R from a ^{60}Co irradiator to block cell division. The medium was then removed and 5,000 endothelial cells were seeded in DMEM with 10% calf serum on top of the test cell feeder layer. The following controls were employed: dishes of irradiated test cells alone, endothelial cells grown on γ-irradiated endothelial cells, and endothelial cells alone grown in the presence and absence of FGF (100 ng ml^{-1}); FGF was added every other day. In some experiments, confluent dishes of test cells were irradiated as described, removed from the dishes by trypsinisation and then seeded at 10,000 cells per dish. This did not alter the results of any experiments. Every other day after the addition of endothelial cells the medium was replaced on all dishes, and duplicate cultures were trypsinised and counted with an electronic cell counter (Electrozone/Celloscope, Elmhurst, Illinois). The number of endothelial cells growing on test cell feeder layers was corrected for the number of cells on dishes of test cells alone. The experiments were conducted five times and representative results are shown. □, Endothelial cells alone (*a*, aortic; *b*, corneal); ■, FGF (100 ng ml^{-1}); ●, γ-irradiated MSV3T3 feeder layer; ○, γ-irradiated BALB/c 3T3 feeder layer; △, γ-irradiated NIH Swiss 3T3 feeder layer; ▲, γ-irradiated endothelial feeder layer (*a*, aortic; *b*, corneal).

Fig. 2 Stained dishes of adult bovine endothelial cells growing on γ-irradiated cells. The experiment was conducted as described in Fig. 1. After 12 days the medium was removed and cells were stained with Diff-Quick (Harleco, Gibbstown, NJ). Control dishes were endothelial cells grown alone in DMEM with 10% calf serum. ABAE, adult bovine aortic endothelial cells; ABCE, adult bovine corneal endothelial cells. *a*, Control; *b*, γ-irradiated NIH Swiss 3T3 feeder layer; *c*, γ-irradiated BALB/c 3T3 feeder layer; *d*, γ-irradiated MSV3T3 feeder layer; *e*, FGF (100 ng ml^{-1}).



tive effects on ABAE cells when compared with γ-irradiated cells (C.R.B. and G.L.N, to be published).

The cloned ABAE cells used in the experiments here have been well characterised as endothelial cells[11]. They can be continuously passaged in tissue culture without loss of endothelial cell characteristics when grown with purified FGF, however, in the absence of FGF they form small colonies of vacuolated, irregularly-shaped cells[11,20]. Our results indicate that vascular ABAE, but not non-vascular ABCE cells, are sensitive to growth factors released into the medium by certain cultured cells. The relationship between these 'factors' and FGF is unknown, although these substances do not stimulate proliferation of ABCE cells which respond to FGF. Recent studies have demonstrated the effects of conditioned medium from various tumour cell lines on primary cell cultures from human umbilical cord endothelium[4] and also the mitogenic properties of tumour cell homogenates on cultured foetal bovine aortic endothelial cells[9]. In both cases, the endothelial cells were mixed populations of uncloned cells and could not be passaged continuously. The cloned ABAE cells[11] should prove useful for endothelial cell proliferation studies, although it is important to note that the control of cell growth *in vitro* may differ from control *in vivo*.

Our findings that untransformed BALB/c 3T3 (A·31), but not untransformed NIH Swiss 3T3, cells significantly stimulate ABAE but not ABCE, cell growth may be related to other observations concerning BALB/c 3T3 cells. When BALB/c 3T3 (A·31) cells adherent to glass[21] or plastic[22] are implanted subcutaneously into BALB/c mice, haemangioendotheliomas[21] or vasoformative sarcomas[22] are formed, and Boone has suggested, on the basis of these experiments, that BALB/c 3T3 (A·31) cells may be preneoplastic. These results, together with morphological studies on BALB/c 3T3 cells[23] show that BALB/c 3T3 cells are probably of endothelial rather than fibroblast origin. BALB/c 3T3 cells possess nerve growth factor[24] and TAF (ref. 5) activities, but it is unclear if the presence of these factors is related to a preneoplastic state. If BALB/c 3T3 cells are endothelial in origin, our results cannot be simply interpreted as a conditioning effect of one type of endothelial cell on another, because ABAE cells have little effect on ABAE cell proliferation (Fig. 1*a*).

The nature of the factor(s) released by BALB/c 3T3 and MSV3T3 cells in our experiments is unknown. It seems to be different from FGF, since FGF but not BALB/c 3T3 or MSV3T3 cells stimulated non-vascular ABCE cells to proliferate (Figs 1*b* and 2). As mentioned earlier, Klagsburn *et al.*[5] have shown that extracts of BALB/c 3T3 cells induce vascularisation when placed on the chorioallantoic membrane of the chicken embryo, whereas BALB/c primary mouse embryo fibroblasts have no effect. We have likewise found that BALB/c 3T3 cells, but not BALB/c primary mouse embryo fibroblasts, will stimulate the proliferation of ABAE cells *in vitro*. It is unlikely that secreted proteolytic enzymes are responsible for the effects of BALB/c 3T3 cells on ABAE cells, since proteolytic enzymes such as thrombin have no effect on ABAE cell proliferation (D. Gospodarowicz, K. S. Brown, C. R. Birdwell and B. Zetter, to be published). Further study will be necessary to ascertain if the reported TAF activity of BALB/c 3T3 cells[5] is responsible for the proliferation of ABAE cells *in vitro*. If this is the case, however, our results are the first to demonstrate that TAF is active *in vitro* as well as *in vivo*.

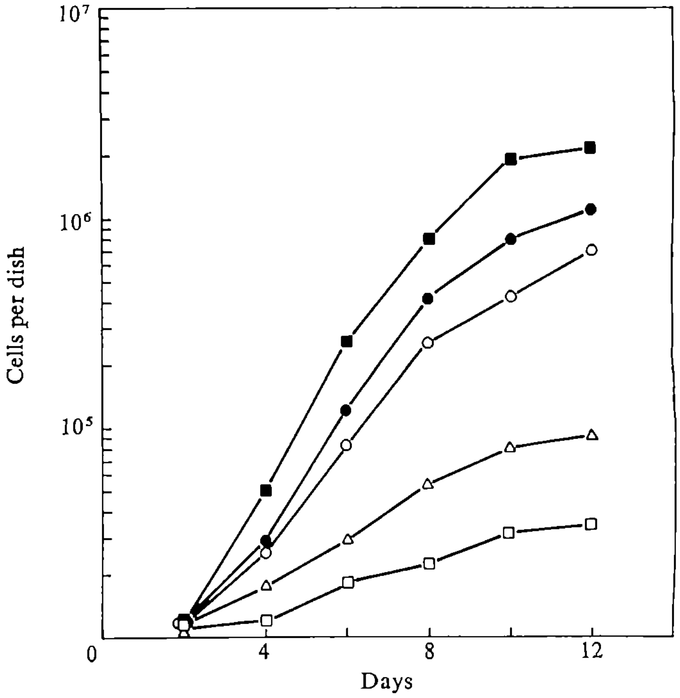


Fig. 3 Growth of adult bovine aortic endothelial cells in conditioned medium. For preparing conditioned medium of BALB/c 3T3 and MSV3T3 cells, 10^5 cells were seeded on to 10-cm dishes in 10 ml of DMEM with 10% calf serum. When the cells were subconfluent (about 4×10^6 cells per dish), the medium was removed, clarified by low-speed centrifugation and filtered through a 0.22-μm filter, then frozen for storage. For preparing conditioned medium of adult bovine aortic endothelial cells, 10^6 cells were seeded as described, since these cells proliferate poorly when seeded at low densities in the absence of FGF. For growth rate studies, adult bovine endothelial cells were seeded at 5,000 cells per 6-cm dish in DMEM with 10% calf serum and after the cells had attached (4–12 h), the medium was replaced with fresh medium or the appropriate conditioned medium. Every other day after the addition of endothelial cells the medium was changed, and duplicate cultures were counted as described in Fig. 1.; FGF was added every other day. The experiments were conducted three times and representative results are shown. ■, FGF (100 ng ml^{-1}); □, DMEM with 10% calf serum; ●, MSV3T3 conditioned medium; ○, BALB/c 3T3 conditioned medium; △ aortic endothelial cell conditioned medium.

Table 1 Effect of various cell lines on the proliferation of adult bovine aortic endothelial cells

Cell line*	% proliferation†
Bovine vascular smooth muscle cells	23
Bovine granulosa cells	18
Bovine corpus luteal cells	31
Bovine corneal epithelial cells	33
Bovine lens epithelial cells	28
Rat 31A ovarian cells	14
C57BL/6J primary embryo fibroblasts	29
BALB/c primary embryo fibroblasts	41

*The cloning and culturing of bovine smooth muscle[11], granulosa[16], corpus luteal[17], corneal epithelial[18], lens epithelial[18], and rat 31A ovary[19] cells have been previously described. Mouse primary embryo fibroblasts were established by trypsinising 16 d-old embryos of C57BL/6J and BALB/c mice, respectively. All cell lines were cultured in DMEM with 10% calf serum (Gibco). The cell lines were tested for proliferative effect as described in Fig. 1, except that cultures were counted only on the twelfth day after the addition of endothelial cells.

†Refers to the percentage increase in the number of endothelial cells on γ-irradiated test cell feeder layers over the number of endothelial cells grown alone in DMEM with 10% calf serum.

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A T cell-replacing factor specific for histocompatibility antigens in mice

COLLABORATION between thymus-derived (T) and non-thymus-derived (B) lymphocytes in immune responses to many antigens has long been established[1]. The actual mechanism of this collaboration, the role of macrophages, and whether it requires contact between the cells or is mediated by soluble factors is still under discussion. Although antigen-specific factors have been reported[2–7], many T-cell replacing factors which have been described are not specific for the antigen used to prime T cells (see ref. 8 for review). Most of these non-specific factors have been obtained from cultures of allogeneic lymphocytes[8–11]. We describe here a specific factor(s) obtained from lymphocytes stimulated *in vitro* by allogeneic cells. This factor replaces helper T-cells in an *in vivo* antibody response to the histocompatibility (H) antigens of the stimulating cells but not to other H antigens or to sheep erythrocytes (SRBC). This is the first description of a factor specific for H antigens which replaces T-cells in T–B collaboration.

Mixed spleen and lymph node cells from BALB/c (H–2^d) mice were stimulated in mixed lymphocyte culture (MLC) by C3H (H–2^k) cells. After 10 d in culture, the alloantigen 'primed' BALB/c lymphocytes were centrifuged and the supernatants were saved (culture supernatant). The cells (90–95 % θ-positive T cells) were washed and resuspended in medium without serum at a concentration of 25×10^6 per ml and incubated at 37 °C for 4 h. The cell-free supernatant was collected after centrifugation (primed cell supernatant) and 0.2 ml was injected intravenously into BALB/c-nu (nude) mice immediately or after storage at −20 °C. After 2 weeks the nude mice were immunised against C3H and C57B1/6 (H–2^b) and tested for their ability to make an antibody response to H–2^k and H–2^b alloantigens. Some nudes were also immunised with SRBC. In this case, mice were immunised first with SRBC since prior immunisation with allogeneic cells has been found to increase the primary antibody response against SRBC (unpublished).

The results of one experiment are presented in Table 1. Nude mice injected with the primed cell supernatant made cytotoxic antibody against C3H cells which were used as stimulators in the MLC. Antibody activity was also detected against CBA cells, which share H–2^k with C3H. In contrast, no antibody activity was detected against C57B1/6 or C3H.SW, a strain congenic with C3H and bearing H–2^b. Nude mice injected with supernatant from unprimed BALB/c cells or those not injected with supernatant did not make an antibody response. One of two nude mice injected with culture supernatant made a low-titred antibody response detected against CBA cells but not with any of the other test cells.

Nude mice given primed cell supernatant made antibody only after immunisation with cells (Table 2). One week after the injection of primed cell supernatant there was no detectable antibody against H–2^k or H–2^b. Following immunisation with cells from both strains, cytotoxic antibody was made to H–2^k only. The titre of the antibody was comparable with that made by normal BALB/c mice. After a second immunisation with C3H and C57B1/6 cells no antibody was made by the nude mice. In contrast, normal BALB/c mice produced higher antibody titres against both H–2 types when boosted.

Table 3 shows the antibody response of nude mice injected with primed cell supernatant or BALB/c lymphocytes and immunised with SRBC. While nude mice reconstituted with BALB/c cells made antibody to SRBC, the primed cell supernatant did not restore the ability of these mice to respond.

These results demonstrate that MLC-primed lymphocytes make a factor(s) which is capable of replacing T cells in the response to H antigens. The antibody response was specific for the H antigens present on the stimulator cell used in the MLC. Thus, factor from BALB/c cells primed against C3H enabled nude mice immunised with cells from C57B1/6 (H–2^b) and C3H (H–2^k) to make antibody which reacted with H–2^k bearing cells but not with H–2^b. The absence of antibody activity to C3H.SW is important since this demonstrates that the antibody responses of nude mice given the primed cell supernatant were restricted to the H–2 region. We do not yet

Table 1 Alloantibody responses of BALB/c-nu mice

Nude mice injected with*	Antibody response to† C3H (H–2^k)	 C57B1/6 (H–2^b)	 CBA (H–2^k)	 C3H.SW (H–2^b)
—	—‡	—	—	—
—	—	—	—	—
Primed cell supernatant	128	—	128	—
	64	—	64	—
	128	—	128	—
	128	—	128	—
Unprimed BALB/c supernatant	—	—	—	—
	—	—	—	—
Culture supernatant	—	—	4	—
	—	—	—	—

*The primed cell supernatant was a cell-free supernatant prepared from MLC-primed BALB/c lymphocytes. 40×10^6 BALB/c cells (spleen and lymph node) were stimulated in MLC against 80×10^6 C3H cells (irradiated with 3,300 rad X ray) in 16 ml culture medium in flasks (Falcon 3024). The culture medium was RPMI 1640 made up to 5 % with human serum, buffered with sodium bicarbonate (2 g l^{-1}) and HEPES (25 mM), and supplemented with L-glutamine (2 mM), L-arginine (0.6 mM), L-asparagine (0.3 mM), penicillin (100 IU ml^{-1}) and streptomycin (100 μg ml^{-1})[21]. After 10 d, the primed cells were washed and resuspended at 25×10^6 per ml in RPMI 1640 without serum and cultured at 37 °C for 4 h. The primed cell supernatant was recovered after centrifugation and used immediately or frozen before use. Unprimed BALB/c supernatant was a cell-free supernatant from freshly isolated, BALB/c lymphocytes (pooled spleen and lymph node) prepared in the same way as the primed cell supernatant. Culture supernatant designates the cell-free culture medium from the MLC. 0.2 ml of primed cell supernatant and cell supernatant from unprimed BALB/c lymphocytes were injected intravenously into nude mice; nude mice injected with culture supernatant were given 0.4 ml.

†Maximal antibody activity was measured on day 10 after immunisation intraperitoneally with 10^7 C3H and 10^7 C57B1/6 spleen cells by complement-dependent cytotoxicity using a two-stage method and absorbed guinea pig serum as a source of complement[21]. Responses are given as one/highest dilution at which antibody activity was detected. Each row represents responses of an individual mouse.

‡No cytotoxic antibody detected at a serum dilution of 1/4, the lowest dilution tested.

Table 2 Primary and secondary antibody responses of BALB/c mice or BALB/c-nu mice injected with primed cell supernatant

Mice*	Antibody response† Pre-immunisation C57B1/6	C3H	Primary response C57B1/6	C3H	Secondary response C57B1/6	C3H
BALB/c	—	—	32	32	128	256
	—	—	32	64	32	128
	—	—	32	64	128	256
	—	—	32	32	128	256
BALB/c-nu + Primed cell supernatant	—	—	—	128	—	—
	—	—	—	64	—	—

*Nude mice were injected with 0.2 ml primed cell supernatant 14 days before immunisation by intraperitoneal injection with 10^7 C57 and 10^7 C3H cells. Each row represents the responses of an individual mouse.

†Given as one/highest dilution of serum giving detectable cytotoxic activity. Pre-immune sera from nude mice were tested on day 7 after injection of primed cell supernatant. The largest primary antibody response was measured between days 7 and 10 after immunisation in BALB/c mice and on day 10 in nude mice. Mice were re-immunised 14 d after the initial immunisation and the secondary response was measured 7 d later.

know the determinants within the H–2 complex against which the antibodies react, but K and/or D determinants are presumably involved since these antisera lysed 100% of lymph node cells.

The lack of T cell-dependent responses to SRBC in mice receiving the primed cell supernatant is of interest since a number of studies have demonstrated that alloantigen-stimulated lymphocytes make nonspecific factors which substitute for T cells in an *in vitro* response to SRBC (refs 8–11). But these factors were normally obtained early in the allogeneic reaction and tested *in vitro*. In our experiments the factor was obtained after 10 d in MLC when the proliferative response was ended and most of the responding (blast) lymphocytes had reverted to small cells. This suggests that, while lymphocytes responding in MLC may release nonspecific factors, elaboration of specific factors is either of a longer duration or requires a longer interval between antigenic stimulation and production of specific factor.

Factor with strong activity was recovered in the primed cell supernatants but not in the culture supernatants. The factor may have been present in the culture supernatants but at too low a concentration to be effective. Alternatively, serum components or other substances released by stimulated lymphocytes could have had an antagonistic effect on the activity of the factor.

Table 3 Antibody response of BALB/c-nu mice to SRBC

	Anti-SRBC haemagglutinating antibody† Primary	Secondary
Untreated controls (2 experiments)	—	—
Nude mice injected with* Primed cell supernatant (4 experiments)	—	—
Culture supernatant (2 experiments)	—	—
BALB/c lymphocytes	3	5
	3	8
	5	8

*Primed cell supernatant (0.2 ml) and culture supernatant (0.4 ml) were injected; 10^7 BALB/c lymphocytes (pooled spleen and lymph node) were injected into control animals.

†Measured as $\log_2$ haemagglutinating activity by 2-mercaptoethanol (2-ME) treated sera, except for the secondary response of the mice injected with supernatant. 2-ME was used because non-reconstituted nudes sometimes make a 2-ME sensitive primary antibody response against SRBC. Antibody responses were measured 7 d after immunisation intraperitoneally with 10^8 SRBC.

The failure to find a secondary response (Table 2) in nude mice injected with the primed cell supernatant argues against a direct effect on precursor T cells known to be present in nude mice[12]. Thus, the factor probably acts directly on specific B cells or has an indirect effect on B cells through macrophages. Conflicting evidence has been presented in the literature concerning the cell type to which antigen-specific factors are directed. Munro and Taussig[13] found that B cells have an acceptor site for antigen-specific factors, suggesting an effect on B cells, whereas Feldmann[14,15] has clearly demonstrated the importance of macrophages in his system.

In our system the nude mice failed to make a detectable antibody response unless challenged with allogeneic cells. Furthermore, the mice were immunised 2 weeks after injection of the primed cell supernatant, demonstrating that the activity of the factor is of long duration. This suggests that antigenic stimulation of specific B cells may not necessarily have to be temporally related to the T-cell help. Such a long term effect of the factor could be due to one of two mechanisms. Either the factor binds directly to specific B cells or to macrophages and is not degraded before antigenic challenge, or the factor gives a signal to the relevant B cells which commits these cells to making antibody on contact with antigen. In the latter case, the signal could result from direct binding to the B cells or after processing by macrophages.

Plate[16] has described a factor which is obtained from MLC activated lymphocytes. This factor substitutes for helper T cells in the generation of cytotoxic T lymphocytes against H antigens. It is not known whether the same T-cell population acts as helpers in antibody production and in the generation of cytotoxic T-cells. But since the specificity of cytotoxic T cells and antibody to major H determinants may be similar[17–20], these systems obviously offer a unique opportunity for dissecting the nature of cell–cell interations in the immune response.

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Magnitude of memory to the major histocompatibility complex

ALTHOUGH the phenomenon of memory is central to the adoptive immune response to antigenic stimulation, the magnitude of the memory effect in graft rejection has never been quantified. We have directly compared the potency of naive and memory cells in two quantitative adoptive transfer assays, one measuring graft rejection and the other graft-versus-host activity. While the well known lack of a significant factor of immunisation in the latter was confirmed, memory cells showed a dramatic, long term increase in their potency to reject heart grafts.

Memory of prior exposure to antigens of the major histocompatibility complex (MHC) was shown many years ago to be both long-lived and specific[1]. Long term memory to these antigens involves a surprisingly small quantitative change in immune responsiveness measured by conventional allo-immune assays, however. Naive animals rapidly reject MHC incompatible grafts and rejection of these grafts is not markedly accelerated by prior sensitisation[2]. The factor of immunisation (FI) in graft-versus-host (GVH) assays is also negligible or absent[3,4]. *In vitro*, although there is an acceleration in the kinetics of the response in mixed lymphocyte reactions, memory cells show no quantitative increase in proliferative capacity[5]. In all other immune responses (including those to weak alloantigens), long term memory is associated with a significant quantitative increase in the response[2,3].

It is possible that the failure to demonstrate memory to strong alloantigens reflects a deficiency in the assay systems used rather than the absence of a specific quantitative change in immune responsiveness in the lymphocytes of a host sensitised to these antigens. Evidence suggesting that this is so derives from adoptive transfer experiments. Sensitised cells differ markedly from naive cells in their capacity to terminate tolerance[6] and to reject long-surviving grafts in immunologically privileged sites[7]. In both cases the response measured is primarily an effector response and not a proliferative one.

We have used a quantitative adoptive allograft assay to compare directly the potency of naive and sensitised cells in effecting the rejection of heterotopic heart grafts in irradiated rats. The same two cell populations were also compared in a quantitative GVH assay to detect any change in proliferative capacity resulting from long term sensitisation. Naive rats of the PVG (Ag-B5), DA (Ag-B4) and Lewis (Ag-B1) strains mutually rejected heterotopic adult heart grafts in 6–8 d (Fig. 1*a*). Previously sensitised animals of these strains showed the trivial acceleration of graft rejection which is expected in respect of major alloantigens (Fig. 1*b*). But, when naive and sensitised cells were compared in an adoptive assay rather than in the whole animal a dramatic increase in potency of sensitised cells was demonstrated. Rats irradiated with 750 rad did not reject Ag-B incompatible hearts for at least 40 d (Fig. 1*h*). Adoptive transfer of syngeneic lymphoid cells to irradiated recipients restored their capacity to reject heart grafts and the degree of restoration was related to the number of cells in the restorative inoculum (Fig. 1*d*–*f*). The minimum number of naive thoracic duct lymphocytes (TDL) or lymph node cells (LNC) which would restore graft rejection to first-set time was 500×10^6 (Fig. 1*d*). But, if the restorative inoculum was obtained from PVG donors bearing long term memory to DA alloantigens as few as 5 to 20×10^6 TDL or LNC caused rejection of DA hearts in first-set time (Fig. 1*j*, *k*). This represents an increase in potency of sensitised cells of 25–100-fold. The increase in potency is specific: PVG cells with memory for DA alloantigen behaved as naive cells in respect to Lewis heart grafts (Fig. 1*g*, *l*). Aliquots of the same cells which showed this dramatic increase in potency in respect of heart graft rejection showed no significant factor of immunisation in GVH responses (Fig. 2).

These results show that prior exposure to antigens of the MHC causes a dramatic and specific increase in potency of the cells responsible for allograft rejection but no increase in the proliferative capacity of the cells responsible for the GVH reaction. This suggests that the subpopulation of T lymphocytes which proliferates in a GVH response and that which mediates tissue damage not only respond to different antigenic determinants[8] and carry distinct cell surface markers[9] but might also respond in quite a different manner to prior sensitisation. The successful demonstration *in vivo* of the dramatic and specific increase in allograft reactivity which occurs in the lymphocyte pool of rats which have been sensitised against antigens of the MHC depends on the use of an adoptive assay. A combination of two factors makes it impossible to demonstrate the extent of this change in the intact rat. It is well established that naive animals have large numbers of antigen sensitive cells responsive to strong alloantigens[10,11]. The experiments reported here showed that there is a physiological limit to the speed with which detectable cell-mediated tissue damage can be effected. If less than 500×10^6 normal cells were given to an irradiated host, rejection time was directly

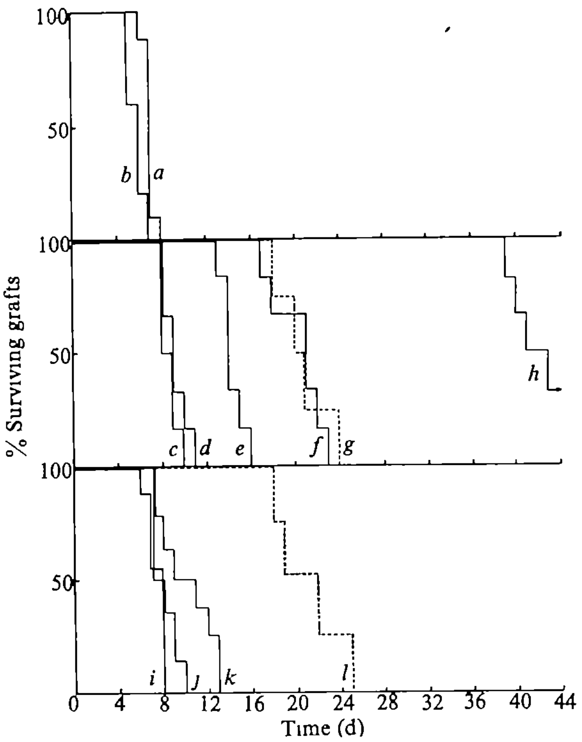

Fig. 1 Life tables of graft survival in PVG rats grafted with DA hearts (solid line) or Lewis hearts (broken line). The aorta and pulmonary arteries of the adult heart grafts were anastomosed to the abdominal aorta and inferior vena cava of the recipient using a modification of the technique of Ono and Lindsey[16]. Rejection was confirmed by loss of electrocardiogram activity. The PVG rats sensitised to DA alloantigens had rejected two sequential DA skin grafts, 6–12 weeks before use. Lines *a* and *b* represent rejection times of DA grafts in normal and sensitised recipients respectively. There is a very small difference in first and second-set rejection times of grafts between Ag-B different strains. *c*–*h* Represent rejection times of grafts in PVG rats given 750 rad on the morning of grafting. Irradiation was from a ^{60}Co source in conditions of maximum back scatter. Within 24 h of grafting groups of these recipients were injected intravenously with graduated doses of LNC from naive PVG rats. Doses of LNC given: *c*, 10^9; *d*, 5×10^8; *e*, 2×10^8; *f*, *g*, 5×10^7. Graft survival was measured from the day of injection with LNC. Irradiated rats given no LNC (*h*) do not reject grafts for 40 d. The minimum number of LNC which will restore first-set rejection was 5×10^8. With smaller numbers of LNC the acceleration of rejection is dose related. *i*–*l* Represent rejection of grafts in irradiated PVG rats injected with LNC from PVG rats sensitised to DA alloantigens. Doses of sensitised LNC given: *i*, *l*, 5×10^7; *j*, 2×10^7; *k*, 5×10^6. Sensitised LNC at a dose of 2×10^7 always restores first-set rejection and at a dose of 5×10^6 restored half the rats to first-set. First-set rejection can be restored to irradiated rats with 25–100-fold fewer sensitised LNC than naive LNC. Specificity of the increased potency of sensitised cells was confirmed by showing that 5×10^7 LNC from rats sensitised to DA (*l*) restored Lewis graft rejection to the same tempo as the same number of naive LNC (*g*).

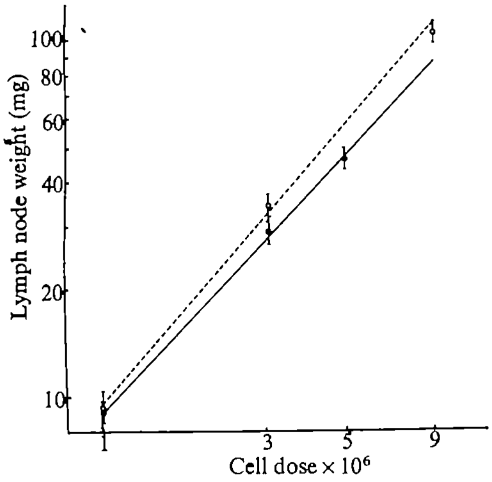

Fig. 2 Comparison of the GVH activity of LNC from normal and sensitised donors using the popliteal lymph node weight assay[17]. Varying numbers of LNC from PVG donors were injected into the right foot pad of groups of (DA×PVG) F_1 recipients matched for age and sex. Log lymph node weight (LNW) is plotted against log cell dose. Each point represents the arithmetic mean of at least 10 LNW. Vertical bars represent the s.e. The calculated regression lines are shown. ●, Response to PVG cells from normal donors (slope 1.0, correlation coefficient 0.94, s.d. of y 0.34). ○, Response to PVG cells from donors sensitised to DA alloantigen (slope 1.1, correlation coefficient 0.93, s.d. of y 0.41). The factor of immunisation at the lowest cell dose was 1.1 and the highest cell dose 1.3.

related to the cell dose (Fig. 1*e*, *f*). But, once this number was exceeded the response was not accelerated by increasing the number of adoptively transferred cells (Fig. 1*c*). 500×10^6 lymphocytes is only a fraction of the total lymphocyte complement of the intact rat[12]. Thus the quantitative increase in cell potency of 25–100-fold which occurs after sensitisation to antigens of the MHC is not detectable in the whole animal because it is already redundant in alloantigen sensitive cells. If, however, lymphocytes are adoptively transferred in limiting dilutions to an animal whose own cells have been destroyed by irradiation, the dramatic quantitative change in the lymphocyte pool of sensitised rats becomes obvious.

It has been shown that both GVH responses[13,14] and skin graft rejection[15] are mediated by pure populations of T cells. The most economical interpretation of our findings is that the clone of memory T cells which mediates graft rejection is functionally expanded 25–100-fold by prior immunisation. Failure of GVH assays to reflect this clonal expansion may indicate that the cells responsible for second set graft rejection are 'end' cells incapable of further proliferation.

Final proof that the increase in potency seen in the cells responsible for graft rejection is determined by antigens of the MHC and not minor histocompatability antigens awaits confirmation in congenic strains. Preliminary experiments indicate, however, that the potency of lymphocyte populations sensitised against multiple minor histocompatability antigens is more than an order of magnitude lower than that of cell populations sensitised against antigens of the MHC.

This dramatic effect of sensitisation on subsequent graft rejection has obvious relevance to clinical transplantation as well as to the fundamental biology of alloimmune responses. Investigations are at present in progress to determine the lifespan and distribution of the T cell responsible for these long term memory effects in the rat.

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Sex steroid hormone regulation of IgA and IgG in rat uterine secretions

SEX steroid hormones may be important in regulating both the systemic and secretory humoral immune system and on the basis of this hypothesis, we have carried out a series of experiments to clarify the action of the sex hormones on the rat uterine immune system. We report here that the stage of the oestrous cycle and the administration of physiological amounts of oestradiol to castrate rats markedly influences the accumulation of immunoglobulin A (IgA) and G (IgG) in uterine secretions. IgG is maximal at pro-oestrus but barely detectable at oestrus, dioestrus, and following ovariectomy. IgA is highest at pro-oestrus, remains elevated at oestrus, but drops to low levels at dioestrus and following ovariectomy. Of the steroids tested only oestradiol stimulated the appearance of IgA and IgG. These findings indicate that oestradiol is important in influencing the presence of both immunoglobulins in the uterus.

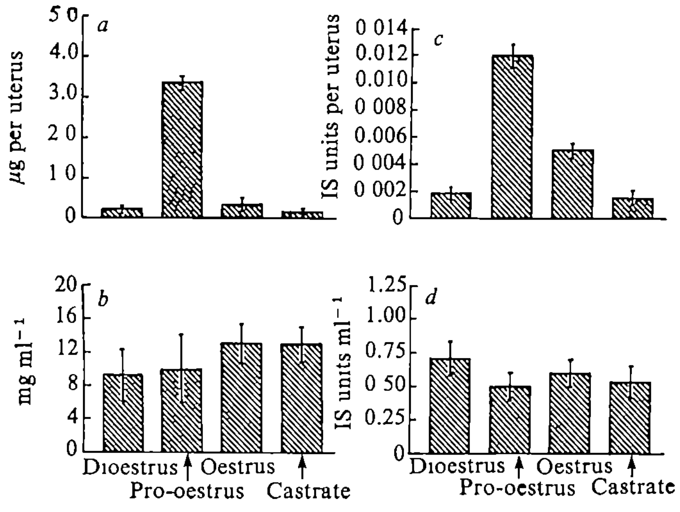
Our findings are of particular interest in the light of the fact that the use of the sex hormones to prevent conception and to reduce the discomfort of menopause has increased the incidence of a number of disorders in women[1–6], including alterations to the systemic immune system. Immunity to viral infections such as chicken pox, herpes simplex and rubella has been shown to be diminished among users of oral contraceptives[6], and women taking oestrogen have been estimated to have a 1.49- to 1.81-fold greater risk of infection than those not taking it. Similar results have been obtained with the administration of sex steroids to rats and mice[7,8]. Reports about the alteration in the local immune system in the female reproductive tract caused by sex steroids are somewhat conflicting. Although immune responsiveness and resistance to genital tract infection vary through the reproductive cycle (refs 9, 10 and 20), levels of IgA and IgG in cervical secretions have been reported by some to remain constant[9] and by others[11,12] to vary during the menstrual cycle. Furthermore, in the absence of apparent infection, oral contraceptives are reported to have altered antibodies in genital tract secretions, but whereas increased levels of immunoglobulins were reported with the use of combination oral contraceptives[9], sharp decreases were observed after the use of the sequential pill[11,12].

Adult female virgin Sprague–Dawley rats (Charles River Breeding Laboratories, Wilmington) maintained in constant-temperature rooms with 12-h intervals of light and darkness were used in this study. Vaginal smears were made daily and females were selected at given stages of the oestrous cycle after at least two consecutive normal 4-d cycles were observed. Ovariectomies were performed 10–15 d before each experiment. Following decapitation of the rats, uteri were perfused with sterile saline through the descending aorta to remove all blood from the vascular beds. Intact uteri were then removed and washed in 2–3 saline baths to eliminate adherent debris. A 26-gauge needle attached to a 1.0-ml tuberculin syringe containing 0.1 ml sterile saline was passed through the uterine wall at the ovarian end. As the saline was introduced into the lumen, a second syringe, with its needle inserted through the cervical musculature, was moved in synchrony to collect the uterine flushing. This procedure was repeated with the second uterine horn using the fluid recovered from the first. The fluid was centrifuged at 15,000*g* for 5 min, after which the supernatant was lyophilised and stored at −20 °C until assayed. Blood was collected immediately after decapitation and was allowed to clot for 30 min at room temperature. Following centrifugation at 15,000*g* for 5 min, serum was removed and frozen at −20 °C until assayed.

IgA and IgG were measured by radioimmunoassay. Rat IgG was purchased from Miles Laboratories; rabbit anti-rat heavy chain IgG antibody was a gift from Dr P. K. Chung (Miles Laboratories, Elkhart, Indiana); IgA-rich serum from rats with IR22 immunocytomas (IgA class) and goat anti-rat IgA (antiserum) prepared against this immunocytoma serum were gifts from Dr H. Bazin (Université Catholique de Louvain, Belgium). Anti-IgG and anti-IgA were coupled to cyanogen bromide-activated microcrystalline cellulose (Sigmacell, Sigma Chemicals, St Louis)[13]. The antigen components of the radioimmunoassay (rat IgG and rat IgA immunocytoma serum) were radiolabelled with ^{125}I using lactoperoxidase-bound Sepharose 4B (ref. 14). Uterine fluids were reconstituted with 50 μl of distilled water just before use and serum samples were diluted 200-fold before assay.

Figure 1*a* shows the levels of IgG in the uterine fluid of rats at various stages in the oestrous cycle and following castration. At pro-oestrus, the time in rats when plasma oestradiol is known to be highest[15], IgG levels were significantly higher (10–15-fold) than those of dioestrus, oestrus, and after castration. No significant differences were measured in serum IgG concentrations (Fig. 1*b*). The corresponding mean values of IgA are shown in Fig. 1*c*. As observed with IgG, IgA is highest at pro-oestrus, but also remains partially elevated at oestrus. Serum IgA levels in these animals show no significant variations (Fig. 1*d*).

Fig. 1 Immunoglobulin G (*a, b*) and A (*c, d*) levels in uterine secretions (*a, c*) and serum (*b, d*) of adult female rats. Values represent the mean ± s.e.m. of 5–8 rats per group. IgG values are expressed as μg per uterus; IgA results as immunocytoma serum (IS) units, where 1.0 IS unit is defined as a concentration of 1 mg of lyophilised immunocytoma serum ml^{-1} distilled water. Values of IgG and IgA at pro-oestrus and IgA at oestrus are significantly different from those measured during dioestrus and following castration ($P \leq 0.01$). No significant differences were measured in serum samples.



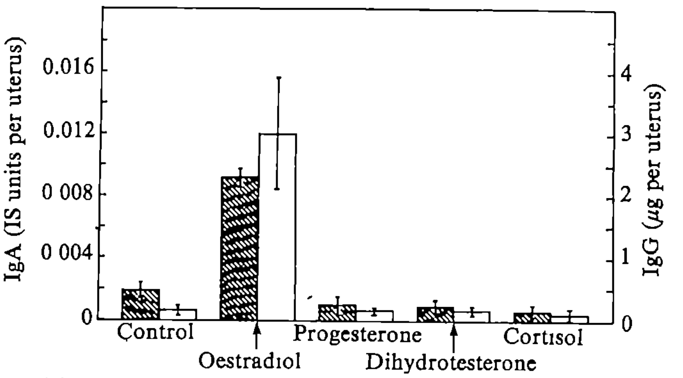


Fig. 2 IgA and IgG in uterine secretions of ovariectomised rats after three daily injections of steroids. Rats were injected with 0.1 ml of ethyl laurate containing either oestradiol (1.0 μg), progesterone (2,000 μg), dihydrotestosterone (1,000 μg) or cortisol (1,000 μg). Control groups received only ethyl laurate. Values represent the mean ± s.e.m. of 5 rats per group. The value for the oestradiol group was significantly higher ($P < 0.01$) than the values of the other groups. Hatched columns, IgA; plain columns, IgG.

Oestradiol, progesterone, cortisol and dihydrotestosterone were administered at concentrations known to elicit physiological responses in their respective target tissues. Time-course studies (not shown) indicated that the antibody response, although detectable at 1 and 2 d, was maximal after 3 d of repeated (daily) injections of steroid. Oestradiol administered daily to castrate rats elevated both uterine IgG and IgA (Fig. 2). Similar results were observed when oestradiol was administered to hypophysectomised rats. In contrast, however, progesterone, cortisol and dihydrotestosterone had no effect.

These results show that uterine IgG and IgA are under oestrogen control and differ from serum levels in the same animals. The data do not enable us to conclude whether the changes observed result from selective transport or *de novo* synthesis of antibodies in this organ. It is known that there are cells in the genital tract mucosa that produce both IgA and IgG. Immunofluorescent labelling of antibodies has led to the identification of subepithelial IgA and IgG plasma cells of the cervix and uterine endometrium[16,17]. In other studies, segments of uterine, cervical and vaginal tissues of the rabbit incorporate radioactive amino acids into IgA and IgG when incubated *in vitro* for 24 h (ref. 18). Similar techniques have also been used to demonstrate that these immunoglobulins are synthesised by the human cervix and vagina[19]. Further studies must be undertaken to determine which mechanism, transport or synthesis, is responsible for the oestrogen-influenced immunoglobulin accumulation in uterine secretions.

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Chemical structure of attachment sites for viruses on human erythrocytes

ENCEPHALOMYOCARDITIS (EMC) virus attaches to erythrocytes from a variety of species through terminal sialic acid residues of the cell surface membrane (M. A. Angel and A.T.H.B., in preparation). Glycophorin, the major sialoglycoprotein of human erythrocytes has been identified as a receptor for influenza virus and some lectins[1]. In this report we present evidence that glycophorin is a human erythrocyte receptor for EMC virus also, and we have tentatively identified the location of a binding site for both EMC and influenza virus on glycophorin.

Glycophorin was prepared by the method of Marchesi and Andrews[1] from human type 0 erythrocytes, the cells used throughout these investigations. Electrophoresis of the glycophorin on 7.5% polyacrylamide gels in the presence of sodium dodecyl sulphate[2] gave a pattern (not shown) similar to that reported by Jackson *et al.*[3]—one major and a few minor components, possibly aggregates of glycophorin, were present, as revealed by staining either with Coomassie brilliant blue or with periodic acid–Schiff reagent.

Glycophorin was recognised as a receptor for influenza virus originally by its ability to inhibit virus haemagglutination[1,3]. We found that both EMC and influenza virus haemagglutination were severely inhibited to about the same extent by glycophorin (Table 1). Influenza virus haemagglutination is inhibited by sialoglycoproteins from many sources[6]. To determine whether or not the inhibitory action of glycophorin was a property of sialoglycoproteins in general, bovine submaxillary gland mucin (Worthington) and human serum glycoprotein (Mann Research) were tested, but neither inhibited EMC virus haemagglutination even at about 10 times the protein concentration at which glycophorin was effective (Table 1). In contrast, influenza virus haemagglutination was inhibited by mucin although not by human serum glycoprotein (Table 1).

Table 1 Effect of glycoproteins on agglutination of human erythrocytes by EMC and influenza viruses

Sialoglycoprotein	Protein concentration* (μg per well)	Haemagglutination titres (% of untreated control) EMC virus	Influenza virus
Human glycophorin	3.7	3	6
Bovine mucin	32.8	100	0.2
Human serum glycoprotein	43.8	100	100

Haemagglutination inhibition tests were performed using a microtitre system (Cooke Engineering) and, as diluent throughout, PBS–G–G, which comprised equal parts of 4.5% (w/v) glucose and PBS containing gelatin to 0.5% (w/v). Serial twofold dilutions of EMC or influenza virus, grown and purified as described previously[2,4], were made in 25-μl volumes and to each haemagglutination tray well was added either 25 μl of the sialoglycoprotein preparation or 25 μl of PBS–G–G in controls. After keeping the mixtures at 4 °C for 1 h, 50 μl of 0.1% (v/v) human erythrocytes were added to each well. The trays were maintained at least 5 h and usually overnight at 4 °C before haemagglutination titres were recorded. The reduction in haemagglutination caused by the sialoglycoprotein treatment is expressed as a percentage of the titre in untreated virus controls.

*Protein concentrations measured by the procedure of Lowry *et al.*[5].

Table 2 Inhibition of EMC virus attachment by glycophorin

(μg protein per sample)	% Attachment
0	75
0.4	35
0.8	23
8.0	0

To 10 μl of ^{3}H-amino acid-labelled EMC virus, prepared as already described[2] was added 10 μl of a glycophorin preparation (10 μl PBS in controls) and after 1 h at ambient temperature, 1 ml of 0.1% (v/v) human erythrocytes in PBS–G–G, (PBS–G–G alone in controls) was added. The samples were maintained a further 1 h at ambient temperature before centrifuging at 3000*g* for 5 min to collect the supernatant fluid. The erythrocytes (or deposits, where present in the absence of erythrocytes) were washed once in PBS–G–G before resuspending in 1 ml of PBS–G–G. After addition of 0.5 ml 30% H_2O_2 to all samples, to bleach the haemoglobin, the radioactive contents of erythrocytes (or deposits), supernatant fluid and wash, were measured in a Beckman LS 250 scintillation spectrometer. % Attachment represents the c.p.m. in the erythrocyte sample compared with the total c.p.m. recovered.

To test whether glycophorin inhibited attachment in addition to the subsequent agglutination step, ^{3}H-amino acid-labelled EMC virus, was mixed with various concentrations of glycophorin before measuring the ability of the virus to bind to erythrocytes. In the absence of glycophorin, 70–80% of the recovered radioactivity was bound by the erythrocytes whereas in the presence of glycophorin (0.8 μg glycophorin protein[5] per sample) this was reduced to 23% (Table 2). At higher glycophorin levels (8.0 μg protein per sample), attachment was virtually eliminated as revealed by recovery of background levels only of radioactivity in the erythrocytes.

Pretreatment of human erythrocytes with neuraminidase reduced EMC virus attachment to about 15% of that of untreated controls and usually abolished haemagglutination by EMC or influenza virus (Table 3). Neuraminidase, previously heated at 100 °C for 10 min, had no such effect, showing that this was an efficient means for inactivating the enzyme. If glycophorin is the molecule to which EMC virus binds on the erythrocyte surface, neuraminidase treatment of glycophorin should abolish its ability to inhibit attachment of EMC virus. This was found to be the case; glycophorin (0.8 μg protein per sample) pretreated with neuraminidase (0.5 units per sample) had almost no inhibitory effect against EMC virus, attachment being 86% of that in the absence of glycophorin (not shown). The loss of inhibitory properties did not result from the heating procedure required to destroy the neuraminidase since glycophorin, heated but not treated with enzyme, reduced EMC virus attachment to 8% and thus showed enhanced inhibitory properties compared with unheated glycophorin at the same concentration (Table 2).

To determine how glycophorin interfered with virus attachment, 25 μl of ^{3}H-labelled EMC virus was mixed with 25 μl of glycophorin, bovine submaxillary gland mucin or human serum glycoprotein (each containing 100 μg protein), or with phosphate-buffered saline (PBS), and after incubating at ambient temperature for 60 min, each sample was examined by sucrose gradient sedimentation. The samples incubated with mucin, human serum glycoprotein or PBS all showed a single peak of radioactivity at a position characteristic of EMC virus, whereas most of the radioactivity in the glycophorin sample sedimented to the bottom of the tube (Fig. 1). A similar result was obtained when each sialoglycoprotein sample was adjusted to the same sialic acid content (12 μg sialic acid per sample as determined by the thiobartituric acid procedure of Warren[7]) rather than the

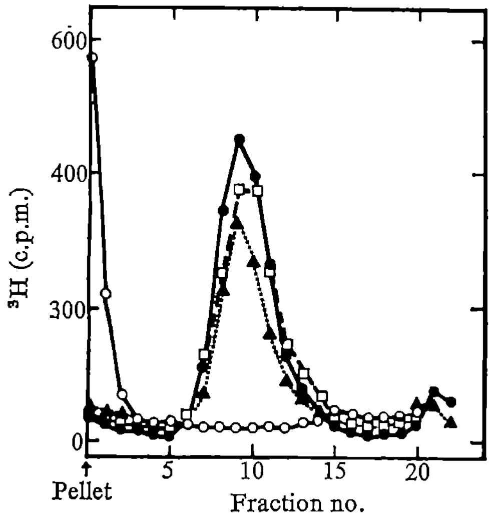

Fig. 1 Rate of zonal sedimentation of ^{3}H-labelled EMC virus incubated for 60 min at ambient temperature with glycophorin (○), mucin (□), humanse rum glycoprotein (●) or PBS (▲) before analysis on 10–30% sucrose gradients containing 0.1 M NaCl and 0.02 M phosphate buffer, pH 8.0 in a Spinco SW 50.1 rotor at 50,000 r.p.m. for 30 min. Direction of sedimentation is from right to left.

same protein content. These results suggest that glycophorin, unlike mucin and human serum glycoprotein, attaches to EMC virus-forming aggregates and in so doing inhibits attachment of virus to cells. However, the aggregates are apparently not sufficiently large to sediment with erythrocytes in the conditions of low-speed centrifugation used to measure attachment of virus to cells.

The work of Marchesi and colleagues[3,8] suggests that trypsin treatment of intact human erythrocytes releases from glycophorin amino acids 1–39, numbered from the N terminus, and leaves projecting from the cell surface a short sequence containing about 25 amino acids beyond which the glycophorin is embedded in the cell surface membrane. We found that trypsin-treated erythrocytes consistently gave haemagglutination titres equal to, or up to threefold dilutions higher for both EMC and influenza virus and bound about the same amount of ^{3}H-labelled EMC virus, compared with controls incubated in the absence of trypsin; typical results are given in Table 3. This suggests that both EMC and influenza virus can attach to the 25 amino acid segment of glycophorin remaining exposed on the cell surface after trypsin treatment. The results do not exclude the possibility, suggested for influenza virus from the work of Marchesi and colleagues[3], that virus attachment sites are also present in the 39 amino acids released by trypsin.

Table 3 Agglutination of enzyme-treated erythrocytes by EMC and influenza viruses

	% of untreated controls		
	Haemagglutination titres		Attachment of ^{3}H-EMC virus
Enzyme treatment	EMC virus	Influenza virus	
Neuraminidase	0	0.3	15.0
Trypsin	200	400	98.0
Trypsin then neuraminidase	2.3	0.3	17.6
Neuraminidase then trypsin	3.1	1.0	ND*

Preparations of 0.1% (v/v) human erythrocytes were incubated at 37 °C for 30 min with 1 mg ml^{-1} trypsin (Worthington) or with 50 units per ml *Vibrio cholerae* neuraminidase (Calbiochem). The enzymes were removed by washing before applying the second enzyme treatment and/or using for haemagglutination or virus attachment studies as described in Table 2.

*ND, Not determined.

The ability of trypsin treated cells to bind ^{3}H-labelled EMC virus or to be agglutinated by either EMC or influenza virus was greatly reduced if the cells were subjected to further incubation with neuraminidase (Table 3). A similar reduction in haemagglutination titre was observed for cells treated with neuraminidase before trypsin (Table 3). These results suggest that sialic acid residues in the fragment remaining with the cells after trypsin treatment are involved in attachment of both EMC and influenza virus to human erythrocytes. Only three amino acids in this fragment have sialic acid attached: serine at positions 44 and 47 and threonine in position 50 (ref. 8). We conclude, therefore, that one or more of these three sialic acid groupings on glycophorin is involved in the binding of EMC or influenza virus by human erythrocytes.

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Synthesis of low molecular weight inhibitor of protein synthesis with enzyme from interferon-treated cells

PROTEIN synthesis in cell-free systems from mouse L cells pretreated with the antiviral agent interferon[1] shows an enhanced sensitivity to inhibition by double-stranded RNA (dsRNA) (refs 2–4). The inhibition of protein synthesis is dependent on incubation with ATP as well as dsRNA and we and others have reported a dsRNA-dependent protein kinase activity(s) in extracts from interferon-treated cells[5–8]. The possible involvement of a viral dsRNA-mediated inhibition of protein synthesis in the sequence of events following virus infection in intact, interferon-treated cells has been discussed previously[2,4]. The situation with extracts from interferon-treated cells[5–8] is reminiscent of that in rabbit reticulocyte lysates in which phosphorylation of an initiation factor by a protein kinase has been implicated in the inhibition of protein synthesis in a variety of conditions including the presence of dsRNA[9–11]. In the interferon-treated L-cell system, however, in addition to the kinase, a heat-stable, low molecular weight inhibitor (LMW inhibitor) of protein synthesis is formed on incubation with ATP and dsRNA (ref. 6). It remained possible from our previous work[6], that the LMW inhibitor might be a small phosphorylated peptide product of the dsRNA-dependent kinase. We show here that this is not the case. In addition, we show that the enzyme responsible for the synthesis of the LMW inhibitor will bind to a column of dsRNA attached to a solid support. It can be eluted in high salt but is relatively unstable in this form. We have used the highly-purified enzyme in its stable column-bound state to synthesise and radioactively label the LMW inhibitor. This synthesis, the partial purification of the inhibitor and its activity in the inhibition of protein synthesis in cell-free systems from L cells and rabbit reticulocytes, are reported here. A more detailed characterisation of the inhibitor is described in the accompanying paper[13].

The enzyme(s) responsible for the synthesis of the LMW inhibitor (inhibitory enzyme) binds to dsRNA, so chromatography on columns of poly(I)·poly(C) bound to Sepharose (poly IC-Sepharose) (refs 14, 15) were used to purify this enzyme(s) and the dsRNA-dependent protein kinase(s). (Prelininary results indicate that the two activities can be separated, A.G.H., unpublished). On passage of a post-ribosomal supernatant fraction from interferon-treated cells (interferon cell sap) through such a column, the inhibitory enzyme and kinase activities were retained. The inhibitory enzyme was recovered on elution with high salt (1 M KCl) at a purification of more than 1,000-fold (a minimum figure is given since the amount of protein in the eluate was too low to be measured). The 1 M KCl eluate contained all of the recoverable inhibitory enzyme activity (⩾25%), assayed either by the formation of the LMW inhibitor on incubation with ATP and dsRNA (refs 5,6), or by its addition with dsRNA to an L cell-free system[4,9]. No such activity was detected with corresponding control cell material.

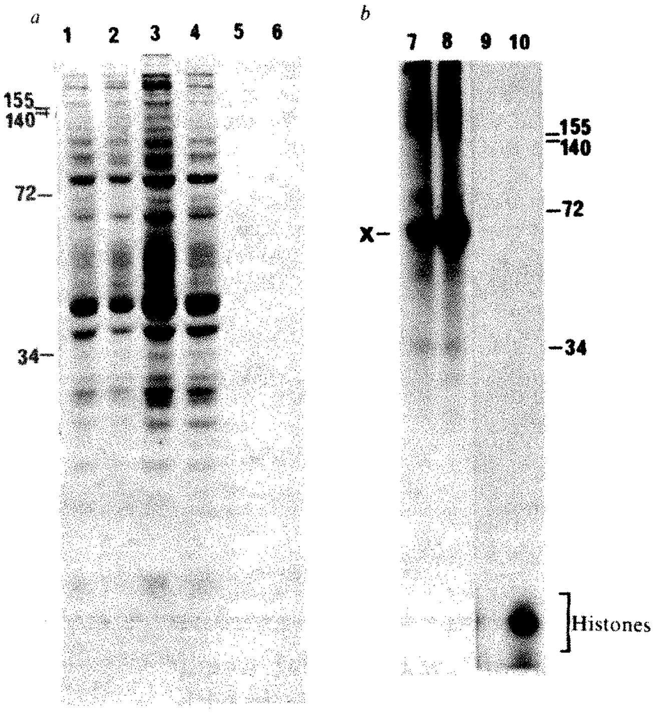

Fig. 1 Chromatography of interferon and control cell saps on poly IC-Sepharose: electrophoretic analysis on polyacrylamide (10%) slab gels containing sodium dodecyl sulphate (SDS) (refs 16, 17). Interferon or control cell caps (50 µl) were passed through columns of poly IC-Sepharose (50 µl) equilibrated with dialysis buffer containing glycerol (DBG: 10 mM HEPES, *p*H 7.5, 90 mM KCl, 1.5 mM magnesium acetate, 7 mM 2-mercaptoethanol and 20% glycerol, v/v). The columns were extensively washed with DBG and the starting material, that retained on the column and the initial pass-through, were analysed for protein and protein kinase activity. *a*, Gel stained for protein: control (1) and interferon (2) cell sap (2 µl), control (3) and interferon (4) pass-through (equivalent to 4 µl of cell sap). For protein retained on the column, the control (5) and interferon (6)-poly IC-Sepharose were heated (95 °C, 10 min) in sample buffer containing SDS (ref. 16), centrifuged and supernatant equivalent to 50 µl of cell sap applied to the gel. *b*, Kinase activity: an autoradiograph of a stained, dried gel is shown. Control (7) and interferon (8) poly IC-Sepharose incubated 60 min at 30 °C with 0.15 mM γ-^{32}P-ATP (7.1 Ci mmol^{-1}, Radiochemical Centre, Amersham) in DBG containing 10 mM magnesium acetate. Phosphorylation of histone: control (9) and interferon (10)-poly IC-Sepharose samples were incubated (60 min, 30 °C) with 1 mM ATP. Calf thymus histone (type IIA, Sigma, 1 mg ml^{-1}) and γ-^{32}P-ATP (0.15 mM, as above) were added and incubation continued for 30 min. The poly IC-Sepharose samples (7–10) were heated (95 °C, 10 min) in sample buffer containing SDS and supernatants equivalent to 7 µl of cell sap applied to the gel. The numbers to the left and right give the molecular weights in thousands of the major reovirus polypeptides λ1, λ2, µ2 and σ3 (ref. 18). The phosphorylated polypeptide band X corresponds to that previously observed on incubation of interferon cell sap with γ-^{32}P-ATP and dsRNA (Figs 1 and 4, ref. 6). Interferon treatment of mouse L cells with low doses (5 to 20 effective units per ml) of highly-purified mouse interferon (⩾5×10^7 units per mg protein), the preparation of cell extracts and of interferon and control cell saps were as described previously[2–4].

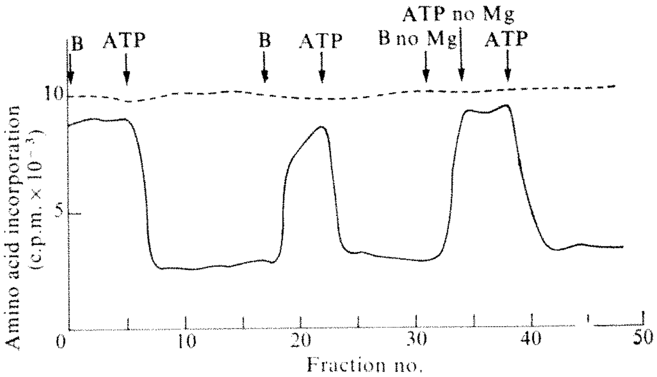

Fig. 2 Synthesis of the LMW inhibitor. Control (- - - - -) and interferon (————) poly IC-Sepharose columns (250 µl) were prepared as in Fig. 1, washed extensively with DBG and incubated at 30 °C in the different conditions indicated (arrows). Abbreviations: B = DBG; ATP = DBG plus 1 mM ATP; B no Mg = DBG minus magnesium acetate; ATP no Mg = DBG plus 1 mM ATP minus magnesium acetate. For each set of conditions the columns were stopped and incubated for 20 min at 30 °C before collection of each 300-µl fraction. Samples diluted 50-fold were assayed for their inhibitory activity on cell-free protein synthesis[4–6,17]. The inhibitory activity of the samples was unaffected by heating at 95 °C for 5 min (data not shown). The ordinate gives the incorporation (c.p.m. per 10 µl sample) of a ^{14}C-amino acid mixture into hot trichloroacetic acid-insoluble polypeptide in response to encephalomyocarditis virus RNA (EMC RNA) in an L cell-free system[4–6,17].

Although it was not possible to detect protein on the interferon and control poly IC-Sepharose columns (prepared as in Fig. 1) by conventional techniques (Fig. 1*a*), when these columns were incubated with γ-^{32}P-ATP phosphorylated polypeptides were detected (Fig. 1*b*). The extent of phosphorylation of a polypeptide of approximately 60,000 in apparent molecular weight (band X, Fig. 1*b*) was the most striking difference between the interferon and control preparations. By analogy with the reticulocyte lysate system[10], this phosphorylation is thought to represent autophosphorylation of the dsRNA-dependent protein kinase[6]. Bands Y and Z reported previously for interferon cell sap (Figs 1 and 4, ref. 6) were not observed here, presumably because the appropriate substrates were not retained on the column. The protein kinase activity(s) of the interferon and control preparations were further investigated by incubation of interferon and control poly IC-Sepharose which had been pre-incubated with unlabelled ATP, with additional histone and γ-^{32}P-ATP. In these conditions histone was phosphorylated with the interferon but not the control poly IC-Sepharose (Fig. 1*b*, channels 9 and 10).

The inhibitory enzyme bound to poly IC-Sepharose was active in the synthesis of the LMW inhibitor. The formation of the inhibitor was dependent on Mg^{2+} and could be controlled by the provision or withdrawal of ATP (Fig. 2). No inhibitor was formed with corresponding control material (Fig. 2). The requirement for ATP suggested that it might form an integral part of the LMW inhibitor, so an attempt was made to label the LMW inhibitor by incubation of the column-bound enzyme with ^{3}H-ATP. In these conditions label (^{3}H) was incorporated into material which co-purified with the LMW inhibitor on chromatography on DEAE–cellulose and Sephadex G-25 (Fig. 3). The behaviour of this column-syn hesised inhibitor on the latter was, incidentally, identical to that previously described for the LMW inhibitor made by incubation of crude interferon cell sap with ATP and dsRNA, being retarded on the column but eluting before marker ATP (Fig. 3, ref. 6). Further evidence that ATP forms an integral part of the inhibitory molecule is presented in the accompanying paper[13].

As here we seemed to be dealing with an inhibitor of a type not previously characterised, it was of interest to see if it had activity in different cell-free systems. The LMW inhibitor, in fact, showed similar activity in the inhibition of endogenous or EMC RNA-directed protein synthesis in the rabbit reticulocyte lysate system, to that observed in the L cell-free system (Fig. 4). The kinetics of inhibition in both systems with the LMW inhibitor were similar to those observed with dsRNA or the haem-controlled repressor in the reticulocyte lysate[9–12,19]. In each case there was a lag of 10–15 min before onset of the inhibition (Fig. 4). In view of this and the effectiveness of the LMW inhibitor at subnanomolar concentrations[13], it remains possible that the LMW inhibitor is not directly inhibitory but that it, in turn, regulates the formation of an inhibitor(s) or subsequent inhibitory events.

We have shown here that the enzyme(s) responsible for the synthesis of the LMW inhibitor and a protein kinase(s) are retained on poly IC-Sepharose. The poly IC-Sepharose column, therefore, provides a possible basis for their separation, characterisation and the determination of their respective roles in the inhibitory process(s). Meanwhile the column technique has provided a simple, convenient method for the synthesis of the LMW inhibitor. The availability of radioactively-labelled inhibitor synthesised in this way has considerably facilitated its initial characterisation[13] and should prove invaluable in the elucidation of its structure, mechanism of action and role in the inhibition of cell-free protein synthesis by dsRNA.

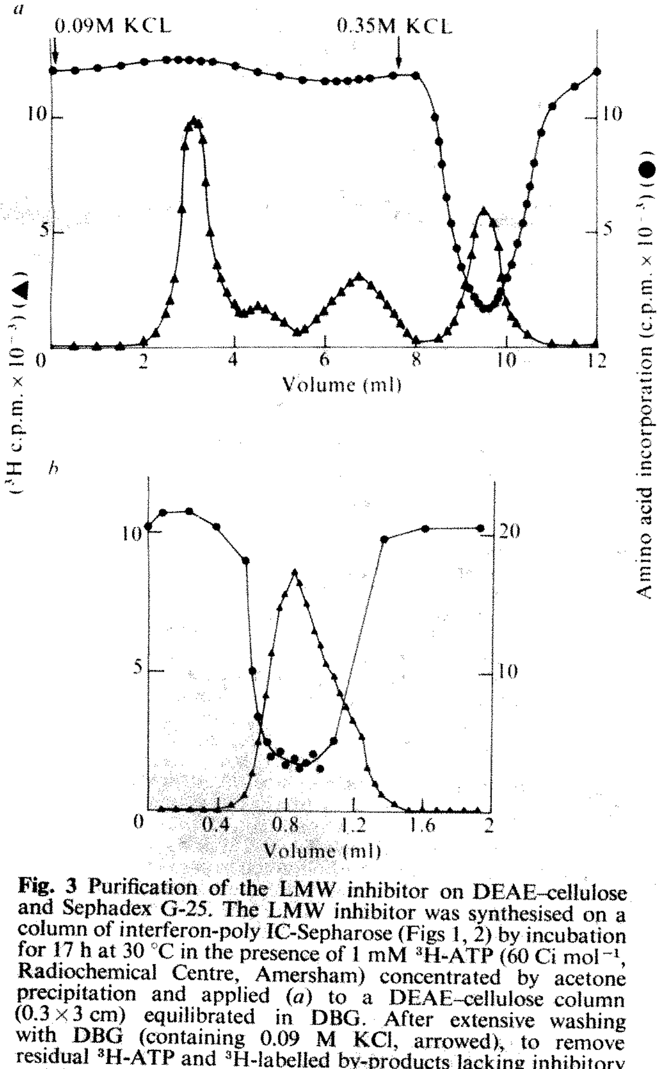

Fig. 3 Purification of the LMW inhibitor on DEAE–cellulose and Sephadex G-25. The LMW inhibitor was synthesised on a column of interferon-poly IC-Sepharose (Figs 1, 2) by incubation for 17 h at 30 °C in the presence of 1 mM ^{3}H-ATP (60 Ci mol^{-1}, Radiochemical Centre, Amersham) concentrated by acetone precipitation and applied (*a*) to a DEAE–cellulose column (0.3 × 3 cm) equilibrated in DBG. After extensive washing with DBG (containing 0.09 M KCl, arrowed), to remove residual ^{3}H-ATP and ^{3}H-labelled by-products lacking inhibitory activity, the LMW inhibitor was eluted at higher salt (0.35 M KCl). Peak fractions containing the LMW inhibitor were pooled, concentrated by acetone precipitation and chromatographed (*b*) on Sephadex G-25 (fine) as described previously[6]. In these conditions excluded material elutes between 0.3 and 0.5 ml and ATP between 0.9 and 1.5 ml (compare with Fig. 3, ref. 6). Samples (at a final dilution of 2,500-fold in *a* and 10,000-fold in *b*) were assayed for inhibitory activity on cell-free protein synthesis[4–6]. The right hand ordinates give the incorporation of ^{14}C-amino acids in an L cell-free system as in Fig. 2 (●). Those on the left show the radioactivity in each fraction (^{3}H c.p.m. per 5-μl sample) originating from the ^{3}H-ATP (▲).

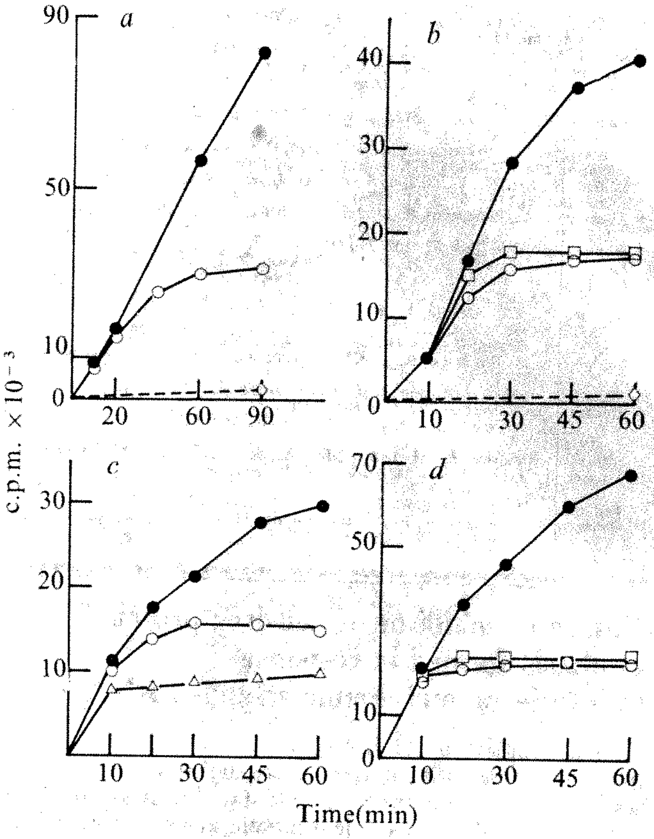

Fig. 4 Assay of the LMW inhibitor in cell-free systems from mouse L cells and rabbit reticulocytes: comparison with dsRNA and the haem-controlled repressor (HCR) (refs 5–8, 19). *a*, *b*, Translation of EMC RNA in the absence (●) or presence of the LMW inhibitor (○) or poly(I)·poly(C) (□) in cell-free systems using: *a*, preincubated and dialysed S10 from mouse L cells[17]. *b*, A micrococcal nuclease-treated rabbit reticulocyte lysate[20]. There was negligible incorporation in the absence of EMC RNA (◇). *c* and *d*, Amino acid incorporation in the absence (●) or presence of the LMW inhibitor (○), poly(I)·poly(C) (□) or HCR (△) in an untreated rabbit reticulocyte lysate system in the absence (*c*) or presence (*d*) of EMC RNA (16 μg ml^{-1}). Preparation of the cell-free systems and the incubation conditions used were based on those described before[17,20,21]. Incubations were at 30 °C for the times indicated (abscissa). The ordinates give the incorporation (c.p.m. per 3 μl sample) of ^{35}S-methionine (200–500 Ci mmol^{-1}, Radiochemical Centre, Amersham) into hot trichloroacetic acid-insoluble polypeptide[17]. The same concentration (1 μl of a 300-fold dilution per 12.5 μl assay) of the same preparation of the LMW inhibitor purified on DEAE–cellulose was used in all cases. (Its inhibitory effect in the reticulocyte lysate was not due to the carryover of poly(I)·poly(C) from the Sepharose column. Any such material was removed by the DEAE–cellulose step[5]. In addition, similar results have been obtained with material further purified on Sephadex G-25 (Fig. 3*b*) and by paper electrophoresis[13].) Poly(I)·poly(C) (Sigma) was added to a final concentration of 100 ng ml^{-1}. The HCR was prepared and used as before[21]. It and the rabbit reticulocyte lysates were gifts of M. J. Clemens.

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Nature of inhibitor of cell-free protein synthesis formed in response to interferon and double-stranded RNA

PROTEIN synthesis in cell-free systems from mouse L-cells pretreated with the antiviral agent interferon[1] shows an enhanced sensitivity to inhibition by double-stranded RNA (dsRNA) (refs 2–4). A dsRNA-dependent protein kinase[5–7] and a heat-stable, low-molecular-weight inhibitor (LMW inhibitor) of protein synthesis which is formed in such systems on incubation with ATP and dsRNA (ref. 7), seem likely to be involved. We have shown[8] that the LMW inhibitor can be conveniently synthesised using a highly-purified enzyme fraction from interferon-treated cells bound to dsRNA on an inert support. Here we report its partial characterisation.

Throughout this work the LMW inhibitor was assayed by its ability to inhibit the translation of encephalomyocarditis virus RNA (EMC RNA) in cell-free systems from mouse L-cells[4,7]. It was synthesised in the presence of ^{3}H-ATP or ATP labelled with ^{32}P in the α or γ position using an enzyme fraction bound to a poly(I)·poly(C)-Sepharose column[8] or, as previously, by incubation of crude cell sap from interferon-treated cells with ATP and dsRNA (refs 4, 7). After chromatography on DEAE–cellulose to remove residual ATP (ref. 8), the different inhibitor preparations were analysed by electrophoresis on paper at *p*H 3.5 (Fig. 1). The inhibitory activity eluted as a single peak (Fig. 1*a*) coincident with the major peaks of ^{3}H, α- or γ-^{32}P (Fig. 1*a–c*). This was true for the intrinsic inhibitory activity of the radioactively-labelled, column-synthesised material or for the unlabelled inhibitor made in crude extracts. It seems, therefore, that the inhibitor synthesised using the column method is not an artefact of the column procedure, but is identical to that formed in response to dsRNA in cell-free systems from interferon-treated cells. Moreover it can be labelled with ^{3}H-ATP or with α- or γ-^{32}P-ATP. The column-bound enzyme[8] is extensively washed before use in the synthesis of the inhibitor. It seems unlikely, therefore, that any low molecular weight component other than ATP (as much as 20% of which is consumed in the formation of the inhibitor) is required in significant amounts for its synthesis.

It is possible to calculate the concentration of AMP in the purified LMW inhibitor preparations from the observed ^{3}H or α-^{32}P counts and the known specific activity of the radioactive ATP used in its synthesis on the column[8]. In four different preparations the dilution of inhibitor required to produce a 50% inhibition of protein synthesis in the cell-free system corresponded to an AMP concentration of 0.3 to 1 nM (Fig. 2). Accepting the intrinsic variability of the cell-free assay and that the inhibitor preparations were not completely pure (contamination ≤ 30%, I.M.K and R.E.B, unpublished results), the correspondence between the four preparations, two labelled with ^{3}H-ATP and two with α-^{32}P-ATP, is remarkably good. Accordingly these data, together with those presented in Fig. 1 and the accompanying paper[8], strongly suggest that ATP is a substrate in the synthesis of the inhibitor and forms an integral part of the biologically active molecule. Further evidence for this and a partial characterisation of the inhibitor was provided by the experiments described in Fig. 3.

Inhibitor was synthesised in the presence of α-^{32}P-ATP, chromatographed on DEAE–cellulose[8] and subjected to enzy-

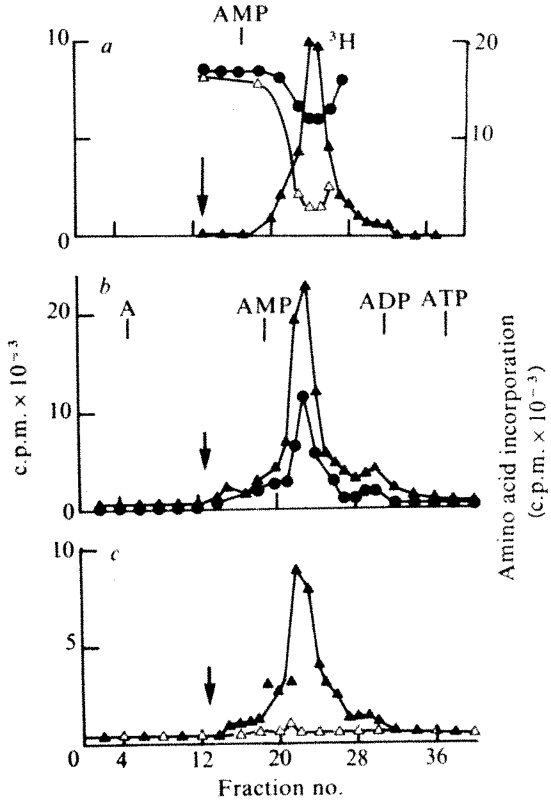


Fig. 1 Electrophoretic analysis of the LMW inhibitor. The LMW inhibitor was synthesised in the presence of ^{3}H-ATP, α- or γ-^{32}P-ATP with an enzyme fraction bound to a column[8], or by incubation of unlabelled ATP with cell sap from interferon-treated cells and dsRNA (refs 4, 7). After chromatography on DEAE–cellulose[8], peak fractions were subjected to electrophoresis on Whatman 3MM paper in pyridine–acetic acid–water (1:10:989, v/v/v) *p*H 3.5 at 4,000 V for 45 min. The paper was dried, cut and 1-cm sections either eluted for assay of inhibitory activity in the cell-free system or counted for radioactivity. In each case the arrow indicates the origin with the cathode to the left. The position to which unlabelled A, AMP, ADP and ATP markers migrated is indicated. (In this system the inhibitor is easily distinguished from any residual ATP). *a*, Inhibitor synthesised in the presence of ^{3}H-ATP (ref. 8) or as previously with cold ATP (refs 4, 7) were electrophoresed in parallel and analysed for radioactivity: (▲, ^{3}H, left-hand ordinate) and for inhibitory activity in the cell-free system. The right-hand ordinate shows the incorporation of a ^{14}C-amino acid mixture into hot trichloroacetic acid-insoluble polypeptide in response to EMC RNA in a cell-free system from control mouse L cells[4,7] (c.p.m. per 10-µl sample) assayed in the presence of eluted material at an overall dilution of 100 (Δ) or 10^{3}-fold (●). There was no inhibition with corresponding control material. *b*, Inhibitor preparations synthesised by the column method[8] in the presence of ●, ^{3}H-ATP and ▲, α-^{32}P-ATP electrophoresed in parallel. *c*, Distribution of ^{32}P in interferon (▲) and corresponding control (Δ) preparations synthesised in the presence of γ-^{32}P-ATP. For the control, interferon cell sap was replaced by control cell sap in the synthetic procedure described in the accompanying paper[8]. Similar controls have yielded similar results with ^{3}H-ATP and α-^{32}P-ATP. For inhibitor synthesised in the presence of ^{3}H-ATP, α- or γ-^{32}P-ATP the intrinsic inhibitory activity migrated with the major peak of radioactivity (data not shown). The ^{3}H-ATP (27 Ci mmol^{-1}) and α and γ-^{32}P-ATP (5 to 15 Ci mmol^{-1}) were obtained from the Radiochemical Centre, Amersham).

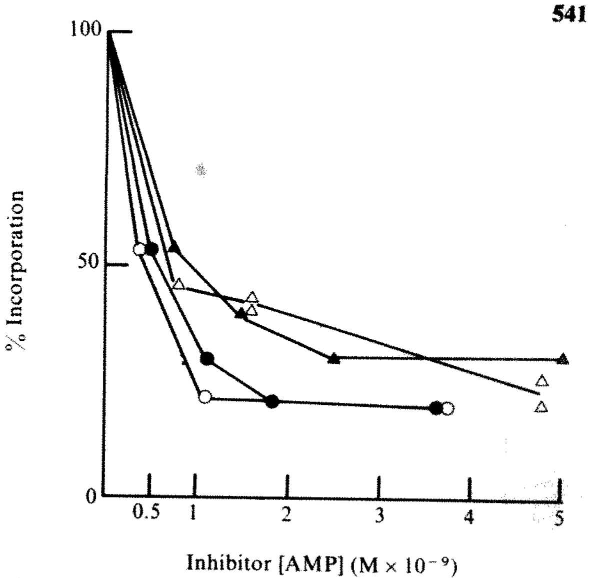
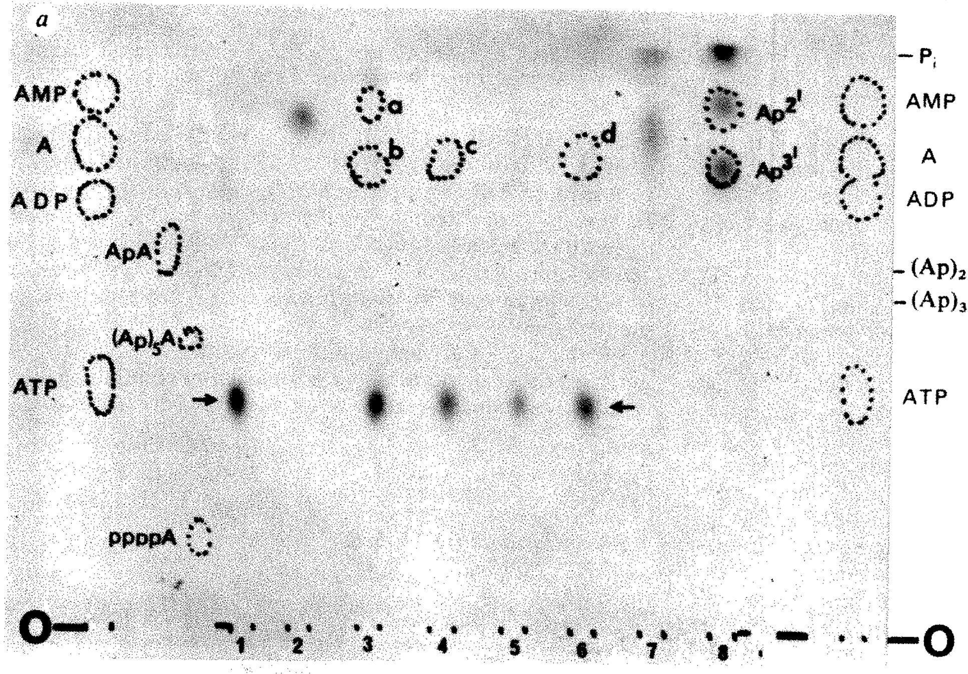


Fig. 2 Concentration of AMP in the inhibitor preparations for 50% inhibition of protein synthesis in the cell-free system. The LMW inhibitor was synthesised by the column method[8] in the presence of 1 mM ATP in a total 'column volume' of 0.15 to 0.9 ml. Radioactive ATP (as in Fig. 1) was added as follows: ^{3}H-ATP to a final specific activity of 145 (○) and 120 (●) Ci mol^{-1}, α-^{32}P-ATP to a final specific activity of 18 (△) and 135 (▲) Ci mol^{-1}. After chromatography on DEAE–cellulose to remove residual ATP (ref. 8) samples containing ^{3}H or ^{32}P were either dried on to glass fibre filters and counted in a Beckman scintillation counter using a toluene-based scintillation fluid with efficiencies of 30% and ⩾ 90% respectively, or assayed at suitable dilution (1 µl of a 1,000–20,000-fold dilution per 12.5-µl assay) for inhibitory activity in the cell-free system. The concentration of AMP in the cell-free system assay (abscissa) was calculated from the known specific activity, the counts in the sample and the dilution used in the assay. Inhibitory activity in the cell-free system was assayed as described in Fig. 1: the ^{14}C-amino acid incorporation for assays in the presence of different dilutions of the inhibitor is expressed as a percentage of the control (no inhibitor) value which varied from 8,000 to 20,000 c.p.m. per 10 µl sample in different experiments.

matic and alkaline digestion. The products were analysed by thin-layer chromatography on polyethyleneimine (PEI) cellulose[9] (Fig. 3*a*). Destruction of inhibitory activity was assayed in the cell-free system[4,7] (Fig. 3*b*). On chromatography the inhibitory activity migrated with the major radioactive component (arrowed Fig. 3*a*). From Fig. 3 it can be seen that the inhibitor is sensitive to digestion with snake venom phosphodiesterase (SVPD), bacterial alkaline phosphatase (BAP) and alkali, but is resistant to micrococcal nuclease, and to nucleases P1, T2 and U2 in conditions of complete digestion of ApA (Fig. 3*a*), (Ap)$_2$A or (Ap)$_5$A included as internal markers. An identical spectrum of resistance and sensitivity to these agents has been obtained with inhibitor labelled with ^{3}H-ATP or γ-^{32}P-ATP and with unlabelled inhibitor formed in crude interferon cell sap[4,7]. (In the latter two cases sensitivity to T2 and U2 has not been tested.) Radioactivity (major spot, arrowed Fig. 3*a*) and inhibitory activity were lost in parallel on partial digestion with alkali or BAP. With γ-^{32}P-ATP-labelled inhibitor, digestion with BAP alone liberated all of the ^{32}P as inorganic phosphate (P$_i$) with corresponding loss of



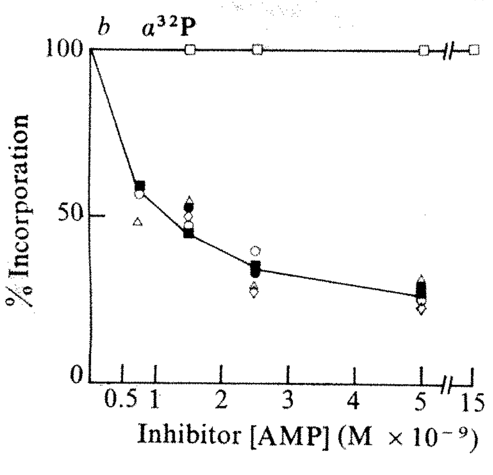


Fig. 3 Enzyme susceptibility of the LMW inhibitor. The LMW inhibitor was synthesised in the presence of α-^{32}P-ATP by the column method and chromatographed on DEAE-cellulose to remove residual ATP (ref. 8). It was subjected to enzymatic and alkali digestion followed either by *a*, chromatographic analysis in 0.75 M potassium phosphate, *p*H 3.4, on thin-layer plates of PEI cellulose[9] (Machery Nagel, Polygram Cel 300 PEI, Camlab), or *b*, assay for inhibitory activity in the cell-free system as described in Figs 1 and 2. The ordinate and abscissa (*b*) were as described in Fig. 2. *a*, 1, Untreated inhibitor; 2–8, inhibitor after digestion with SVPD, P1, T2, micrococcal and U2 nucleases, BAP and BAP followed by alkali, respectively. *b*, ■, Untreated inhibitor; △, ○, ●, ◇, inhibitor after digestion with P1, T2, U2 and micrococcal nucleases, respectively; □, inhibitor after digestion with SVPD, BAP or BAP plus alkali. (Similar results were obtained for biological activity after digestion with alkali alone, data not shown.) Enzyme digestions were carried out in 5–20 µl reactions for 1–6 h at 37 °C in the conditions described by Brownlee[10] (for T2, U2 and micrococcal nucleases, SVPD and BAP) and Rose[11] (for P1). Alkali digestion was for 17 h in 0.3 M KOH. An autoradiograph of the dried plate is shown (*a*). In *a*, 3, 4 and 6 an approximately 100-fold excess of unlabelled ApA was added to the α-^{32}P-labelled inhibitor before digestion to provide a positive control showing enzyme activity. Spots a, b, c and d were detected under ultraviolet light and are the digestion products of the ApA—a, p(5′)A; b, A; c, e, A and A(3′)p. Digestion was without effect on the inhibitor (arrowed) here or in parallel experiments in which the same digestions were carried out in the absence of ApA (data not shown). The slight variation in the intensity of the inhibitor spot (arrowed) reflects variation in the amounts loaded. The optical density markers (dotted circles) A, AMP, ADP, ATP, Ap (mixture of 2′ and 3′), A(3′)p, ApA and p$_4$A were from Sigma and (Ap($_2$A and (Ap)$_3$A from Boehringer. (Ap)$_5$A and micrococcal nuclease were from PL Biochemicals. The SVPD and P1 (from *Penicillium* citrinum), T2 (RNase T2 from *Aspergillus*)[10] and U2 (RNase U2 from *Ustilago*)[10] nucleases were gifts of Dr Beverley Griffin. BAP was 'BAPF' from Worthington.

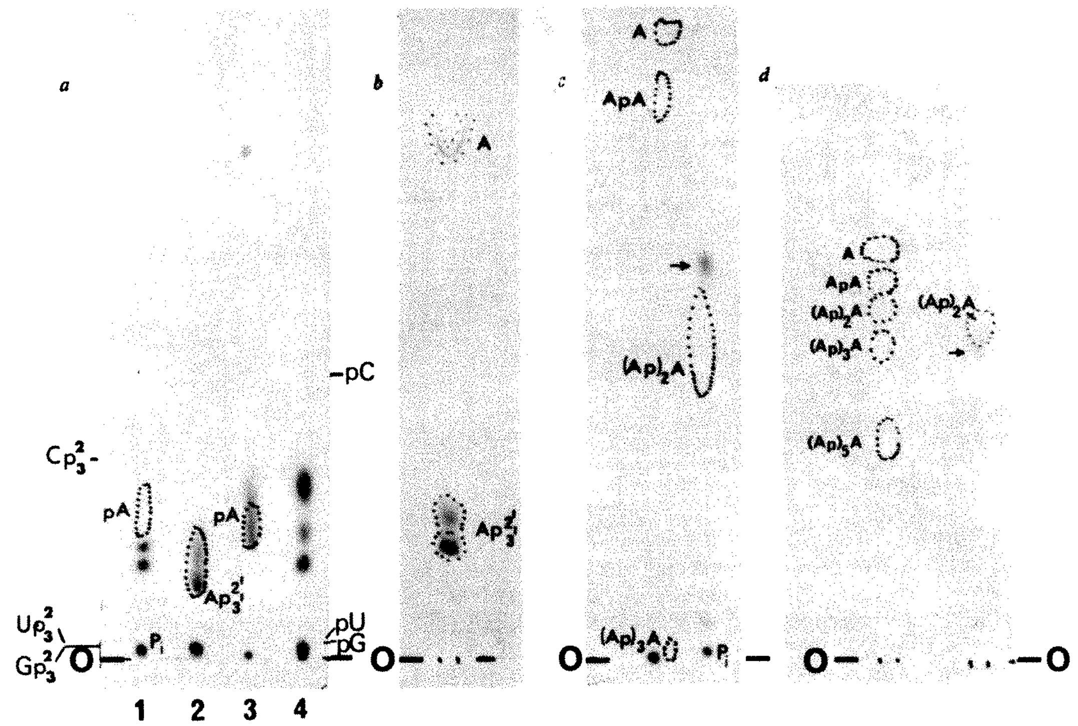


Fig. 4 Analysis of the products of SVPD, BAP and BAP followed by alkali digestion of the ^3H or α-^{32}P-labelled inhibitor. LMW inhibitor was prepared and subjected to enzymatic and alkali digestion as described in Fig. 3, before further chromatographic analysis of the digestion products, *a*, α-^{32}P-labelled inhibitor: 1 and 2, alkali digest of BAP-treated inhibitor with unlabelled pA or Ap (mix of 2′ and 3′), respectively, as markers. 3, SVPD digest with unlabelled pA marker. 4, Mixture of alkali digest of BAP-treated inhibitor plus SVPD digest of untreated inhibitor. *b*, ^3H-labelled inhibitor: alkali digest of BAP-treated inhibitor. *c*, *d*, α-^{32}P-labelled inhibitor: BAP resistant core (arrowed). Thin-layer chromatography was in 1 M acetic acid on PEI cellulose[12] for *a*, *b* and *c*: for *d* it was in *n*-propanol–concentrated ammonia–water (55:10:35, v/v/v) on cellulose (Polygram Cel 300, Machery Nagel, Camlab, Cambridge, England)[13]. Optical density markers (dotted circles) were as in Fig. 3. Detection of radioactivity was by autoradiography in *a*, *c* and *d* and by fluorography[14] in *b*.

biological activity. It seems reasonable to conclude that the major radioactive component (arrow Fig. 3*a*) is indeed the biologically active inhibitor. Accordingly, a more detailed analysis of its digestion products was undertaken to obtain further information concerning its structure.

Digestion of the α-^{32}P or ^3H-labelled inhibitor with SVPD yielded 5′ AMP as the only major product (Fig. 3*a* track 2, and Fig. 4*a* track 3 for ^{32}P: data for ^3H not presented). Sequential degradation with BAP and alkali yielded $^{32}P_i$, 2′-AMP and 3′ AMP (in approximately equal amounts, Fig. 3*a* track 8 and Fig. 4*a* tracks 1 and 2) and ^3H-labelled adenosine, 2′ AMP and 3′ AMP (Fig. 4*b*). The BAP-resistant core of the molecule, however, is not simply ApApA. It migrates to a point intermediate between $(Ap)_2A$ and $(Ap)_3A$ on chromatography in a system which separates according to size (Fig. 4*d*) and migrates with neither of these markers when chromatographed on PEI cellulose with 0.75 M potassium phosphate *p*H 3.4 (Fig. 3*a*) or acetic acid (Fig. 4*c*) solvents. Moreover, even after removal of all BAP-sensitive phosphate the α-^{32}P or ^3H-labelled 'BAP core' of the inhibitor remains resistant to, for example, P1 and T2 nucleases in conditions of complete digestion of ApA or $(Ap)_2A$ included in the same reaction mix (data not presented). The possible involvement of pA residues linked 2′–5′ rather than 3′–5′ has not been tested. Overall it seems unlikely from these results that the LMW inhibitor corresponds directly to the possible 'pleiotypic activator' A(5′)pppp(5′)A (ref. 15), or to the various analogues of the 5′-terminal cap structures of messenger RNA (ref 16). These latter, for example, seem to be required at much higher concentration ($\geq 10\ \mu M$) than the LMW inhibitor to be effective in the inhibition of cell-free protein synthesis[17–20].

From our previous work it remained possible that the heat-stable LMW inhibitor might be a small phosphorylated polypeptide product of the dsRNA-dependent protein kinase present in extracts from interferon-treated cells. This is not the case. Here we have shown that although the inhibitor is resistant to a variety of nucleases it is sensitive to digestion with SVPD, BAP and alkali and can be labelled with ^3H and α-^{32}P-ATP as well as with γ-^{32}P-ATP. It apparently contains two or more AMP residues in SVPD and alkali-labile linkage. Its precise nature remains to be determined, but the results suggest that it is a 'di- or oligonucleotide' of unusual or modified structure which is effective at subnanomolar concentrations in the inhibition of protein synthesis in cell-free systems from mouse L-cells or rabbit reticulocytes.

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Effects of sodium butyrate on synthesis of human chorionic gonadotrophin in trophoblastic and non-trophoblastic tumours

SOME human tumours and tumour-derived cell lines are capable of producing 'eutopic' peptide hormones and other proteins that are normally synthesised by the tissue from which the tumour originated. For example, choriocarcinoma cells, which are derived from cancer of the placental trophoblast, can synthesise human chorionic gonadotrophin (hCG) (refs 1, 2), and cultured pituitary adenomas can produce growth hormone (GH) (ref. 3). On the other hand, it is well established that a variety of malignant human tumours can produce 'ectopic' peptide hormones and other proteins which are not normally associated with the tissue of origin[4–8]. Ectopic synthesis of placental proteins, including the placental isoenzyme of alkaline phosphatase as well as the hormones hCG and human placental lactogen, may provide specific markers for neoplasm, since these proteins are not normally found in measurable amounts in the serum of men or of non-pregnant women[9]. Several human glycoprotein trophic hormones, including hCG, thyroid-stimulating hormone (TSH), luteinising hormone (LH), and follicle-stimulating hormone (FSH), are composed of two non-identical subunits, α and β (refs 10–13). These four hormones have virtually identical α subunits; in contrast each has a unique β subunit, which is responsible for biological specificity[14]. Complete hCG and free subunits are secreted by

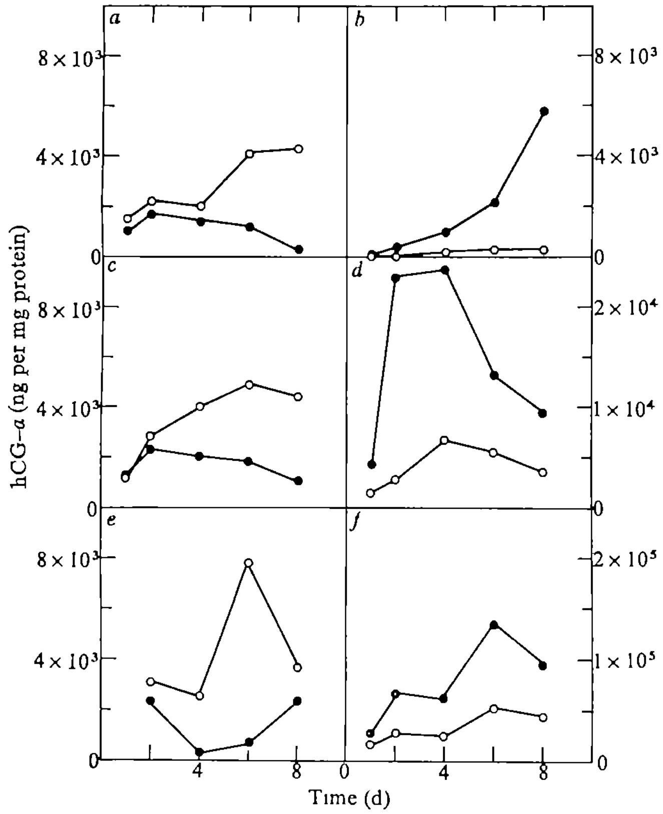


Fig. 1 Effect of sodium butyrate on hCG-α synthesis. *a*, JEG-3; *b*, HeLa CCL2; *c*, Reid; *d*, HeLa S$_3$ *e*, BeWo; *f*, ChaGo. Cultures were grown at 37 °C in 25-cm^2 flasks in 5 ml of α MEM (BeWo, ChaGo and HeLa cells) or Ham's F-12 (JEG-3 and Reid) supplemented with 10% foetal bovine serum. Media and serum were obtained from Flow Laboratories, Rockville, Maryland. Media were replaced every 2 d. HCG-α and hCG concentrations in the culture medium were measured by a specific double-antibody radioimmunoassay using hCG-α and hCG as reference standards[15]. For measurement of cellular protein content, cells were collected by removing the medium, washing the cells twice with saline, and scraping the cells off the plastic surface with a rubber policeman. The cells were collected by centrifugation and then frozen. The frozen pellet was resuspended in 0.3 ml buffered saline (0.9% NaCl in 0.01 M Tris-HCl, *p*H 7.4), and the cells were ruptured by sonication (Raytheon Magnetostrictive Oscillator, Model DF-101, Raytheon, Manchester, New Hampshire). The sonicates were centrifuged at 10,000g for 15 min. Total protein in the supernatant solutions was determined by the method of Lowry *et al*[19]. ○, Control; ●, 2 mM sodium butyrate.

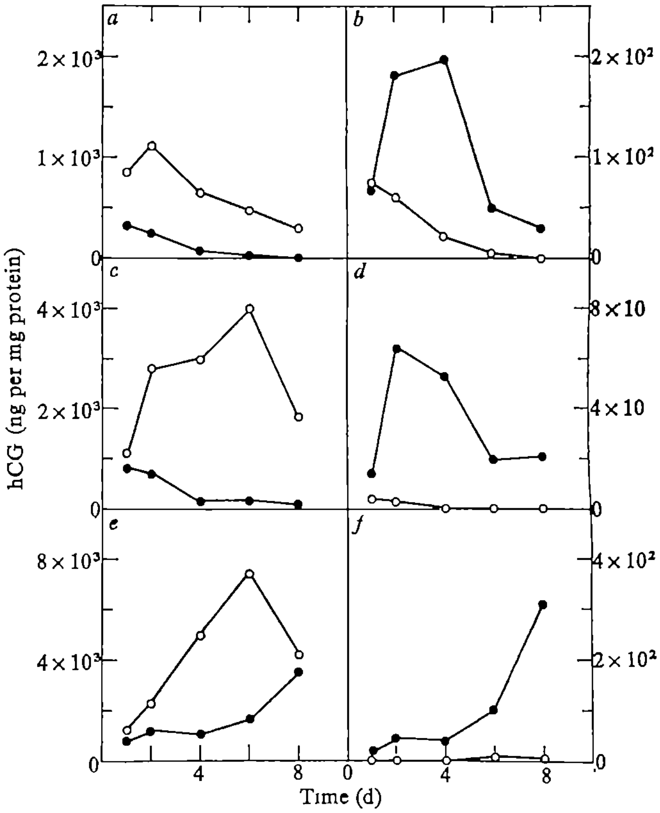
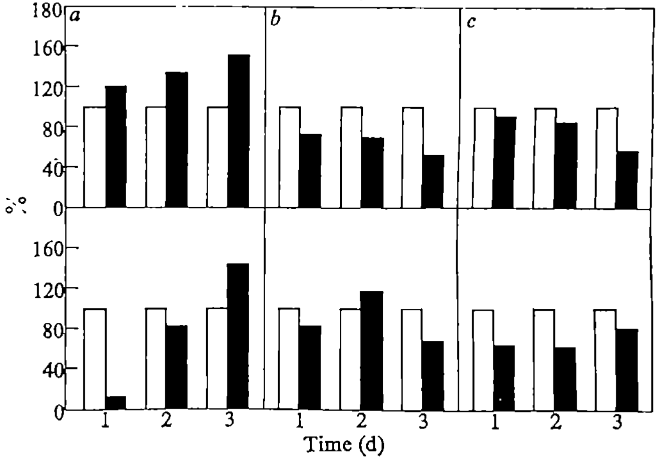


Fig. 2 Effect of sodium butyrate on hCG synthesis. *a–f* And conditions as in Fig. 1. ○, Control; ●, 2 mM sodium butyrate.

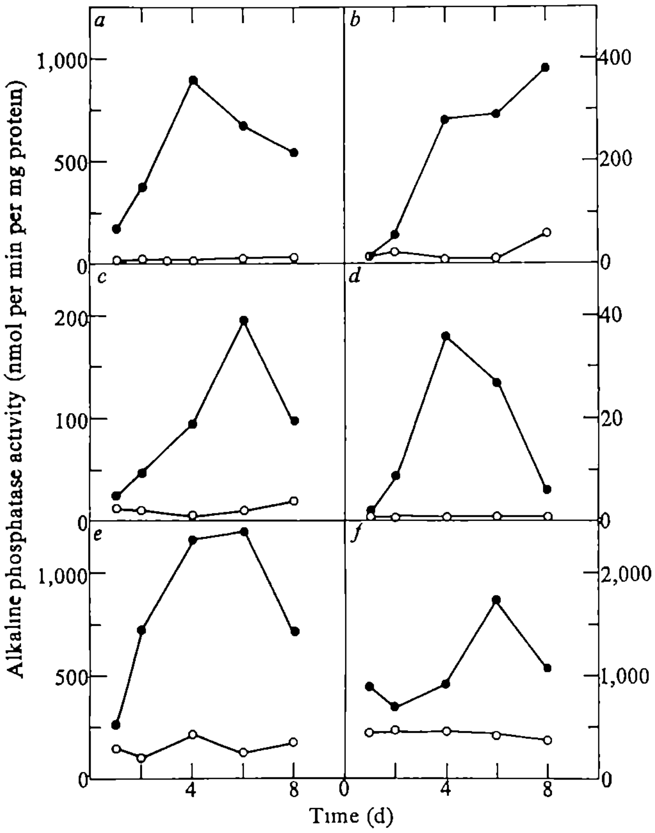
Fig. 3 Effect of sodium butyrate on the rate of synthesis of cell DNA, RNA and protein. Monolayers of JEG-3 (top row) or HeLa S$_3$ cells (bottom row) were incubated with medium containing 2 mM sodium butyrate. At indicated times, cells were labelled with (*a*) methyl-^{3}H-thymidine (5 μCi ml^{-1}), (*b*) ^{3}H-uridine (5 μCi ml^{-1}), or (*c*) ^{3}H-leucine (5 μCi ml^{-1}), for 1 h at 37 °C (radioisotopes were purchased from New England Nuclear, Boston, Massachusetts). After removal of the radioactive medium, the cultures were washed with phosphate-buffered saline, and the cells were lysed with 0.01 M Tris-HCl, *p*H 7.5, containing 0.6% SDS and 10 mM EDTA. Macromolecules in samples of the lysates were precipitated with an equal volume of 10% cold TCA. The precipitates were collected on GFC glass fibre filter disk (Reeve Angel Laboratory, Clifton, New Jersey) and washed extensively with 5% TCA. The filters were dried and the radioactivities were measured. Data are expressed as percentages of control values. Open bars, control; solid bars, sodium butyrate.



normal and neoplastic placenta[1,2,14] and by many non-trophoblastic tumours[15,16]. Since hCG is a eutopic hormone for trophoblastic tumours, but an ectopic hormone for non-trophoblastic tumours, it occured to us that its synthesis by the two types of tumours might be regulated differently. We therefore compared the synthesis of hCG and hCG-α by three choriocarcinoma cell lines—BeWo (ref. 1), JEG-3 (ref. 2) and Reid (P. O. Kohler, unpublished observations)—with the synthesis by two non-trophoblastic tumour-derived cell lines—ChaGo and HeLa. (ChaGo was derived from an hCG-producing pulmonary carcinoma[16], and HeLa from a carcinoma of the cervix[17].) We also tested the effects of sodium butyrate on the synthesis, since this compound had been shown to induce hCG-α and hCG synthesis in certain HeLa cells[18]. We found that in the absence of inducer, all of the cell lines can produce hCG-α—the trophoblastic tumour cells at roughly comparable rate, but the non-trophoblastic tumour cells at widely different rates (Fig. 1).

The basal production of complete hCG by the non-trophoblastic tumour cells is only marginal compared with its synthesis by the choriocarcinoma cells (Fig. 2). Sodium butyrate has strikingly different effects on the two classes of tumour cells: it induces synthesis of hCG-α and hCG in the non-trophoblastic tumour cells, but represses it in the trophoblastic tumour cells (Figs 1 and 2). Because of its differential effects on synthesis, we thought it possible that sodium butyrate might be more toxic for the choriocarcinoma cells. This is unlikely, however, for two reasons. Sodium butyrate inhibited the incorporation of uridine and leucine at roughly the same degree in JEG-3 and HeLa S$_3$ cells (Fig. 3). Although sodium butyrate slightly enhanced the incorporation of thymidine in JEG-3 cells but transiently inhibited the incorporation in HeLa S$_3$ cells, the long term effects seemed to be the same for both types of tumour cells (Fig. 3). The placental isoenzyme of alkaline phosphatase was induced by this compound in all cell lines examined (Fig. 4). Furthermore, since intracellular and extracellular hCG-α and hCG are affected to the same extent by sodium butyrate (data not given), the observed reduction of these proteins in choriocarcinoma cells and induction in non-trophoblastic tumour cells are not due to its effects on hormone secretion.

Fig. 4 Effect of sodium butyrate on alkaline phosphate activity. *a–f* and conditions as in Fig. 1. Alkaline phosphatase activity in the sonicates was measured by release of *p*-nitrophenol from *p*-nitrophenyl phosphate at *p*H 10.7 and 37 °C (ref. 20). ○, Control; ●, 2 mM sodium butyrate.



Our data indicate that the regulation of hCG and hCG-α production differs in placental and non-placental tumours. Detailed analysis of the molecular mechanisms underlying the biosynthesis of this peptide hormone by these cell lines may yield insight into the essential nature of ectopic protein production.

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Potent prolactin and growth hormone releasing activity of more analogues of Met-enkephalin

MORPHINE stimulates prolactin and growth hormone secretion[1,2], so the identification of brain peptides with opiate-like activity (methionine-enkephalin and leucine-enkephalin)[3] raised the possibility that these peptides, beside their role as modulators of pain[4], could also be involved in the control of neuroendocrine functions. We did in fact find that Met-enkephalin administered intraventricularly led to a stimulation of prolactin and growth hormone release[5–7]. β-Endorphin (β-LPH$_{61-91}$) was, however, found to be 500 to 2,000 times more potent than Met-enkephalin (β-LPH$_{61-65}$) in stimulating the release of the pituitary hormones. Since both peptides show similar affinity for the opiate receptor when binding studies are performed at 0 °C in conditions of minimal enzymatic degradation[8,9], the markedly different biological activities of the two peptides after intracerebral injection are likely to be due to a more rapid inactivation of Met-enkephalin. We show here that the two analogues resistant to enzymatic degradation, [D-Ala^2]Met-enkephalin and [D-Ala^2]Met-enkephalinamide can stimulate prolactin and growth hormone release after intraventricular injection at a level of potency which is 500 to 5,000 times higher than that of the natural Met-enkephalin molecule.

Since both plasma[10,11] and brain tissue[11] contain high levels of enzymatic activity hydrolysing the Tyr^1–Gly^2 bond as the initial step of inactivation of Met-enkephalin, we first tested

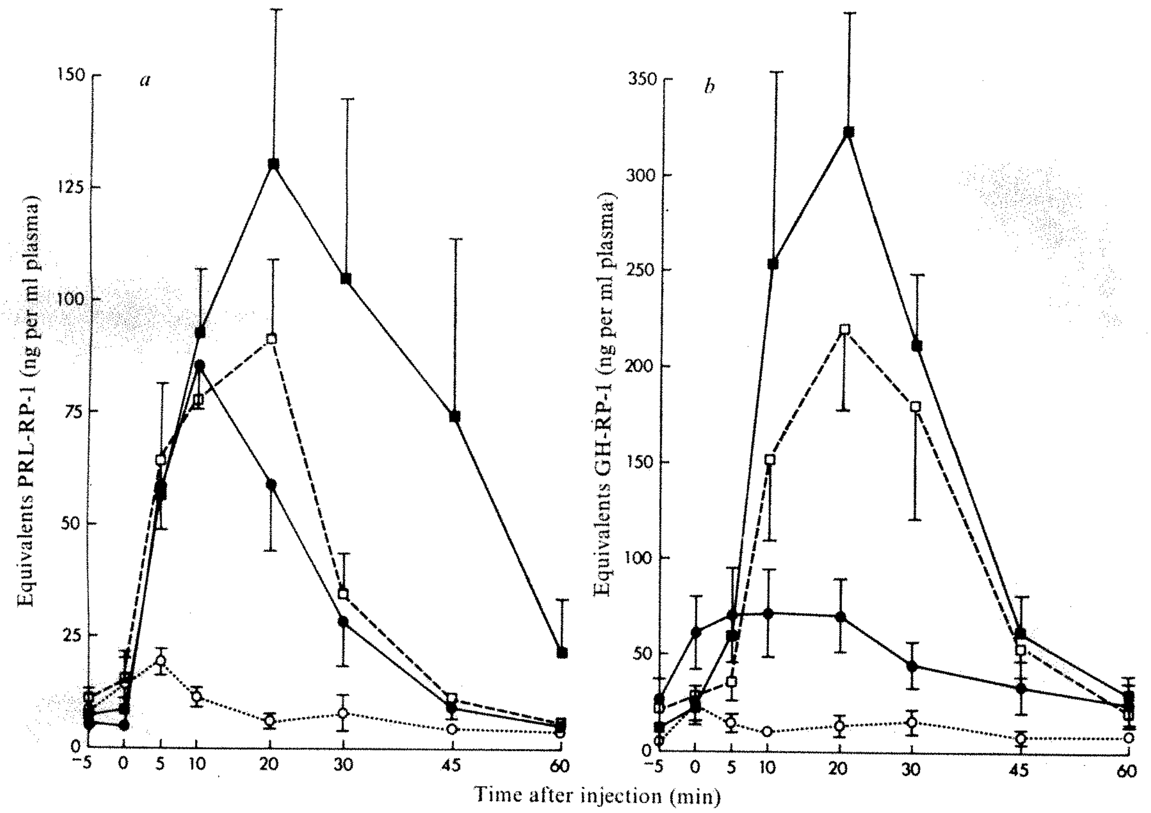

Fig. 1 Effect of increasing doses of Tyr–D–Ala–Gly–Phe–Met ([D-Ala^2]Met-enkephalin) on plasma prolactin (*a*) and growth hormone (*b*) levels in the rat. To minimise stress-induced inhibition of growth hormone (GH) and stimulation of prolactin (PRL) release, adult male Sprague–Dawley rats (225–250 g) were handled twice a day for 7–10 d before insertion of a catheter into the right superior vena cava under Surital (50 mg per kg body weight, i.p.) anaesthesia. The animals were then implanted stereotaxically with a stainless steel cannula (25 gauge) in the left lateral ventricle. Two days later, animals were injected through the intrajugular catheter with 0.5 ml of sheep anti-somatostatin serum 5 min before injection of the peptides to be tested which were injected in a volume of 40 µl of 0.9% NaCl over a 3-min period in freely-moving animals. Blood samples of 0.7 ml were then withdrawn at the indicated time intervals for measurement of plasma GH and PRL by double-antibody radioimmunoassays using materials provided by NIAMDD. Data are expressed as mean ± s.e.m. of triplicate determinations. Correct placement of the intracerebral cannulae was confirmed by injection of 10 µl of methylene blue at the time animals were killed. The peptides used were synthesised by solid-phase methods and purified as before[12]. ○, Control; ●, □, ■; [D-Ala^2]Met-enkephalin at 50, 150 and 300 µg respectively.

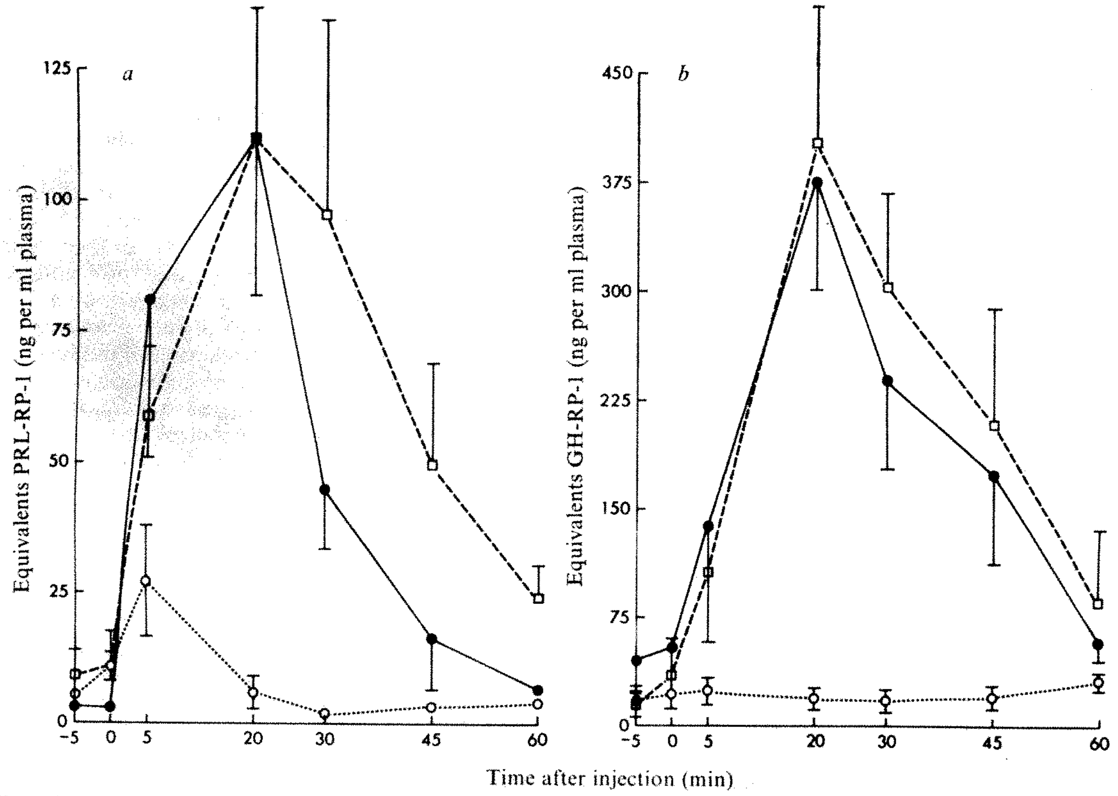

Fig. 2 Effect of intraventricular injection of 62 or 200 µg of [D-Ala^2]Met-enkephalinamide on plasma prolactin (*a*) and growth hormone (*b*) levels in the rat. The experiment was performed in conscious freely-moving adult male animals bearing chronic intraventricular and intrajugular cannulae as for Fig. 1. ○, Control; ●, 62.5 µg and □, 200 µg [D-Ala^2]Met-enkephalinamide.

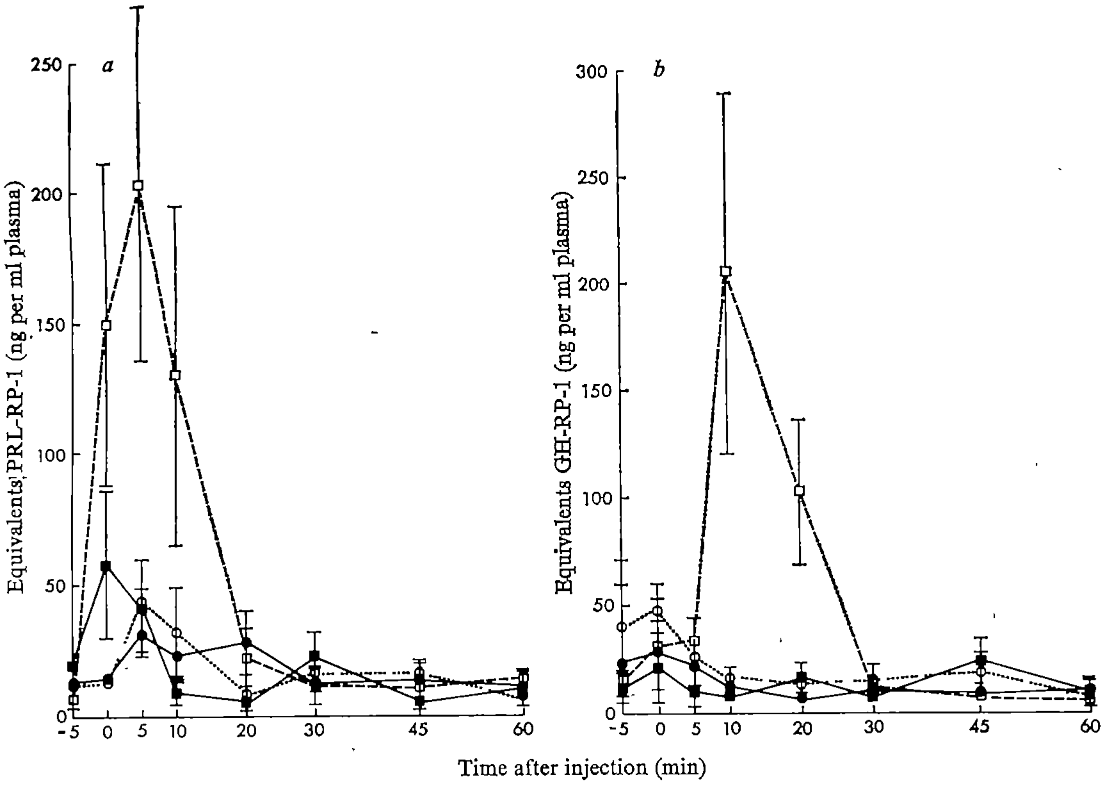

Fig. 3 Effect of intraventricular injection of 400 μg of *N*-acetyl [D-Ala^2]Met-enkephalin (●), Met-enkephalin-ethylamide (□) or [D-Tyr^1]Met-enkephalin (■) on plasma prolactin (*a*) and growth hormone (*b*) levels in the rat. ○, Control. The experiment was performed in conscious freely-moving adult male animals bearing chronic intraventricular and intrajugular cannulae as described in Fig. 1.

the growth hormone and prolactin releasing activity of analogues of Met-enkephalin that seemed to be resistant to degradation, retained their activity for the opiate receptor[8,9] and were found to be potent agonists in the vas deferens assay[12].

The minimal effective dose of Met-enkephalin on growth hormone and prolactin release after intraventricular injection in the rat is approximately 400 μg (refs 5–7), but it can be seen in Fig. 1 that [D-Ala^2]Met-enkephalin has a maximal stimulatory effect on prolactin and growth hormone release at the doses of 50 and 150 μg, respectively. As observed previously with Met-enkephalin and β-endorphin[5–7,20] the stimulatory effect of [D-Ala^2]Met-enkephalin is exerted at a lower dose and earlier after its injection on prolactin than growth hormone release. In fact, while an important stimulatory effect of the Met-enkephalin analogue on plasma prolactin levels was seen 5 min after its injection, the effect on plasma growth hormone levels was detected 5 min later.

Substitution of D amino acids in position 6 of the decapeptide luteinising hormone-releasing hormone (LHRH) leads to peptides with much greater potency and duration of action[13,14] while replacement of the tryptophan residue at position 8 in somatostatin by its D-isomer enhances the growth hormone-release inhibiting activity of the molecule approximately eightfold[15]. Our data show that substitution of the glycine residue of Met-enkephalin by D-alanine leads to a dramatic increase of the growth hormone and prolactin releasing potency of the peptide. Figure 1 indicates that [D-Ala^2]Met-enkephalin given by intraventricular injection is 1,000 to 2,000 times more potent than Met-enkephalin itself.

Met-enkephalinamide has been reported to be slightly more effective than Met-enkephalin in an opiate receptor assay[9,16], so it is interesting to note than [D-Ala^2]Met-enkephalinamide (Fig. 2) is about five times more potent than [D-Ala^2]Met-enkephalin as stimulator of growth hormone and prolactin release. In fact, while maximal stimulation of prolactin and growth hormone release is obtained with [D-Ala^2]Met-enkephalin at the 300-μg dose, a similar effect was obtained with 62.5 μg of [D-Ala^2]Met-enkephalinamide, a peptide at least 5,000 times more potent than Met-enkephalin in this assay.

In agreement with our receptor binding studies[9,12] and vas deferens bioassays[12], [D-Tyr^1]Met-enkephalin and *N*-acetyl Met-enkephalin did not show any growth hormone or prolactin releasing activity at the 400 μg dose, thus indicating the importance of the L-tyrosine residue for biological activity of the molecule. Substitution of the C-terminal glycine-amide residue in the LHRH molecule by ethylamide led to an analogue 10 to 15 times more potent than the natural hormone[17]. It is interesting to note that addition of the ethylamide group at the COOH-terminus of Met-enkephalin markedly increased its growth hormone and prolactin releasing activities (Fig. 3).

Our data show that two Met-enkephalin analogues studied have an activity comparable with that of β-endorphin itself as stimulators of growth hormone and prolactin release after intraventricular injection. These analogues should be useful for study of the physiological role of endogenous opiate peptides, not only as analgesics[18] and behavioural modulators[19] but also in the control of neuroendocrine functions. These data and previous work[5–7,20] in fact suggest that endogenous opiate-like peptides are involved in the control of growth hormone and prolactin secretion. The model described here could also be useful for precise assessment of the biological activity of endogenous and synthetic opiate-like substances.

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A synthetic enkephalin analogue with prolonged parenteral and oral analgesic activity

METHIONINE and leucine enkephalin, the morphinomimetic pentapeptides from mammalian brain[1-3] produce a weak and short-lived analgesia following intracerebroventricular (i.c.v) or intravenous (i.v.) administration to mice[4] and rats[5]. β-endorphin[6,7], another morphinomimetic fragment of β-lipotropin but with 31 amino acid residues, shares its amino-terminal sequence with met-enkephalin. In addition to showing marked and long-lived analgesic activity following i.c.v. and i.v. administration[8-10], the pronounced effects of β-endorphin have led to the suggestion that it may be implicated in the aetiology of mental illness[11-13]. We report here a number of the pharmacological properties of five synthetic pentapeptides structurally related to met-enkephalin, two of which exhibit significant analgesic activity after oral administration and which are more potent analgesics administered parenterally than β-endorphin.

The enkephalin analogues were prepared by fragment condensation methods. Analgesia was estimated in the mouse (tail flick[14] and hot plate[15,16] procedures), the rat (paw pressure test based on the technique described by Randall and Selitto[17]) and the rhesus monkey (shock titration test[18-20]). The affinity for opiate receptors was assessed by the inhibition of ^{3}H-naloxone binding to rat brain membranes using a method described previously[4].

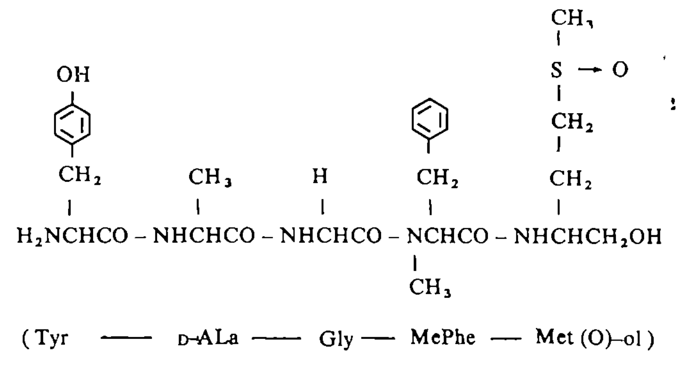
OH
CH$_3$
S → O
CH$_2$
CH$_2$
CH$_2$
CH$_3$
H
CH$_2$
CH$_2$
H$_2$NCHCO – NHCHCO – NHCHCO – NCHCO – NHCHCH$_2$OH
CH$_3$
(Tyr —— D-ALa —— Gly —— MePhe —— Met (O)–ol)

Fig. 1 Structural formula of 33–824

In the tail flick test (Table 1), met-enkephalin exhibited short-lasting analgesia following i.c.v. and i.v. administration[4]. A gradual increase of analgesic potency and duration after i.c.v. administration resulted from the alteration of the carboxyl group to the carbinol analogue (Met^5-ol), the substitution of the glycine residue in position 2 by D-alanine (D-Ala^2,-Met^5), and the combination of these two modifications (D-Ala^2,Met^5-ol). The latter derivative showed subcutaneous as well as prolonged i.c.v. and i.v. analgesic activity. In contrast, the corresponding (D-Ala^2,Met^5) enkephalin amide[11] was only active following i.c.v. administration. Oxidation to the sulphoxide (D-Ala^{2}Met(O)^5-ol) and *N*-methylation of the phenylalanine residue gave compound FK 33–824 (referred to throughout as 33-824), (D-Ala^2,MePhe^4, Met(O)^5-ol)-enkephalin (Fig. 1), which produced definite analgesic activity even after oral administration. 33-824 was, on a molar basis, not only about 30,000 and 1,000 times more potent than met-enkephalin and morphine respectively after i.c.v. administration, but also 23 times as active as β-endorphin.

In binding studies using ^{3}H-naloxone[22] and rat brain membranes (Table 1) met-enkephalin and its Met^5-ol derivative had a greater affinity for the opiate receptor in the presence of the enzyme inhibitor[23] bacitracin than in its absence, indicating that even in this *in vitro* system, both compounds are subjected to inactivation. For all the other compounds including morphine, the dose–response curves for opiate-receptor binding were not affected or were shifted only slightly to the right, suggesting that these compounds are sufficiently stable in the *in vitro* conditions used. In agreement with the *in vivo* results,

Table 1 Analgesic activity in the mouse and inhibition of ^{3}H-naloxone binding to rat brain membranes

Compound	Tail-flick test ED$_{50}$					Relative molar dose i.c.v.	IC$_{50}$ (nM) of specific ^{3}H-naloxone binding	
	i.c.v. 15 min (μg per mouse)	i.v. 15 s (mg kg^{-1})	i.v. 30 min (mg kg^{-1})	s.c. 30 min (mg kg^{-1})	p.o. 120 min (mg kg^{-1})		Without bacitracin	Bacitracin (0.1 mg ml^{-1})
Met-enkephalin	68*	153				1	412	138
Met^5-ol	43	52				0.6	50	27
D-Ala^2, Met^5	0.7	51				0.01	61	132
D-Ala^2, Met^5 -ol	0.04	12	13	59	> 640	0.0006	20	31
D-Ala^2, Met(O)^5–ol	0.01	12	3.5	3.9	319	0.0001	60	95
D-Ala^2, MePhe^4, Met-(O)^5-ol (33-824)	0.002	0.7	0.4	1.4	102	0.00003	12	14
β-Endorphin	0.3					0.0007	35	35
Morphine-HCl	1.0		1.8†	3.0	20.5‡	0.03	73	83

*At 2 min; †at 15 min; ‡at 30 min.

The ED$_{50}$ value[33] for the analgesic activity in the tail-flick test is defined as the dose that prolonged the reaction time (in s) by more than 75% in half the animals. Ten animals were used per dose. All doses refer to the free base. The IC$_{50}$ is the concentration of substance required to inhibit by 50% the specific binding of ^{3}H-naloxone (1 nM) to pre-incubated (30 min at 37 °C) membranes of rat brain minus cerebellum in 0.05 M Tris buffer (*p*H 7.4 at 25 °C). Five concentrations of each compound were incubated in triplicate at 25 °C for 40 min in the presence of 100 mM NaCl (refs 4, 25). Filtrations were performed at ı25 °C. Specific binding is the difference between ^{3}H-naloxone binding in the absence and presence of 1 μM levallorphan. Values are the mean of at least three determinations with s.e.m. of less than 15%.

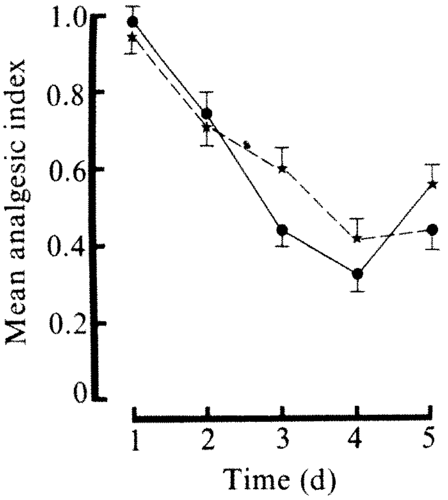

Fig. 2 Mean analgesic index (± s.e.) recorded in the paw pressure test in the rat[28] following s.c. administration of morphine (7.5 mg kg^{-1}, ●) and 33–824 (3.2 mg kg^{-1}, ★) twice daily for 4 d. On day 5, 33–824 (3.2 mg kg^{-1} s.c.) was given to the morphine-pretreated rats and morphine (7.5 mg kg^{-1} s.c.) to the 33–824-pretreated animals. Twenty rats were in each group. The reaction thresholds were recorded immediately before and 30 min after each injection (0815 h and 1545 h).

33–824 displayed the highest affinity for the opiate receptor. The data in Table 1 indicate that in the absence of bacitracin an approximately 30-fold increase in affinity was observed between the least (met-enkephalin) and the most (33-824) potent compound in this series. This increase is much smaller than the dramatic, graded increase observed *in vivo*. The 'sodium response ratio' for 33-824, calculated from the ID$_{50}$ values obtained in the presence and the absence (data not shown) of NaCl (ref. 24), was 8.2, thereby placing it in the same range as the partial morphine agonist pentazocine which had a ratio of 7.1 (ref. 4). Since there is *in vivo* no evidence to suggest that 33-824 possesses morphine antagonistic properties, the value of the 'sodium response ratio' as a predictor[24] of the degree of morphine-like activity of opioid peptides remains uncertain[25].

In a more detailed comparison of the analgesic properties of 33-824 and morphine (Table 2), 33-824 was about twice as potent subcutaneously in the mouse and rat. Thirty minutes after oral administration, however, 33-824 was 5 (tail flick) and 3.5 times (hot plate) less active than morphine. The similarity of the ED$_{50}$s obtained with 33-824 in the inflamed and non-inflamed paws in the paw pressure test confirm the central nature of its analgesic effect. Aspirin, with a predominantly peripheral analgesic effect[26], only affects the threshold in the inflamed paw. In the rhesus monkey, 33-824 was parenterally about 2–3 times less potent than morphine, depending on the route of administration. Following rectal administration, however, 33-824 was active whereas no analgesic effect was observed with morphine. The degree and duration of analgesia with 33-824 was dose dependent in all species investigated. In the mouse (tail flick) the ED$_{50}$s after i.v. administration at 0.5 and 2 h were 0.4 and 0.8 mg kg^{-1} respectively. Orally, in the same test, the ED$_{50}$ increased between 2 and 3 h after administration from 102 to 114 mg kg^{-1}. In the rhesus monkey the duration of action following i.v. administration of the minimal effective dose was at least 5 h.

The parallel displacement to the right of the analgesic dose–response curves of 33-824 and morphine in the tail-flick test in a manner similar to that obtained with methionine enkephalin[4] following pretreatment with naloxone (0.56 mg kg^{-1} i.v.), provide evidence for the morphine-like qualities of 33-824. This was confirmed by the ability of 33-824 to produce mydriasis and a Straub tail response in the mouse, that were abolished by naloxone administration. Repeated administration of 33-824 to rats twice daily for four consecutive days[27] resulted in the development of tolerance to its analgesic effect in the paw pressure test and cross-tolerance with the analgesic effect of morphine given on day 5 (Fig. 2). In rats similarly pretreated with morphine, 33-824 exhibited a markedly reduced analgesic effect (Fig. 2).

In the single dose suppression test[28] in morphine-dependent rhesus monkeys (3.2 or 5.6 mg kg^{-1} i.v. 6 times daily for a minimum of 4 weeks), 33-824 (1.8–5.6 mg kg^{-1} i.v.) abolished the morphine withdrawal syndrome. Substitution of morphine injections in morphine-dependent, non-withdrawn rhesus monkeys by 33-824 (1.8 mg kg^{-1} i.v. at 4 h intervals for 24 h) prevented the appearance of the morphine withdrawal syndrome. In a self-administration study in three male, drug-naive rhesus monkeys[29], 33-824 (0.01 and 0.032 mg kg^{-1} i.v. per injection), was not self-administered in spite of continual access to the drug for a total of 4 weeks. When the concentration was increased to 0.1 mg kg^{-1} i.v. per injection, all three animals started to exhibit self-administration behaviour which increased gradually over a period of 5 weeks, until the mean daily amount self administered was about 13 mg kg^{-1} i.v. (Table 3). Naloxone (i.v.) produced a marked withdrawal syndrome resembling that observed following opiate withdrawal and saline substitution for 33-824 injections resulted in all three monkeys in the occurrence of a marked opiate-like withdrawal syndrome lasting 2–3 d and accompanied during the first 48 h by an increase in lever-pressing activity (Table 3).

33-824 (10 mg kg^{-1} i.p.) was devoid of morphine-antagonistic properties in the mouse as, in contrast to naloxone (0.56 mg kg^{-1} i.p.), it did not produce jumping behaviour in morphine-dependent[30] mice. Thus, 33-824 may be regarded at this stage as being a pure morphinomimetic drug without any antagonistic properties.

33-824 (10 μg per rabbit) administered into the 4th ventricle produced a marked catatonia characterised by muscular rigidity and immobility lasting at least 2 h and which had the same outward appearance as that exhibited by β-endorphin in rats[11,12]. Morphine (1 mg per rabbit in 0.1 ml) injected directly into the 4th ventricle caused marked respiratory depression

Table 2 Analgesic activity of 33–824 and morphine

Compound	ED$_{50}$(mg kg^{-1}) Tail flick (mouse)		Hot plate* (mouse)		Paw pressure† (rat) inflamed paw	non-inflamed paw	Minimal effective dose (mg kg^{-1}) Shock titration (rhesus monkey)		
	s.c.	oral	s.c.	oral	s.c.	s.c.	i.v.	s.c.	rectal
33–824	1.4	102	1.1	20.5	0.7	0.9	1.8	1.8	56
Morphine-HCl	3.0	20.5	2.1	5.8	1.3	2.0	1.0	0.56	>100

*At 60 min; † at 30 min.

ED$_{50}$ values[33] for the analgesic activity in the tail-flick, hot plate, and paw pressure tests are defined as the doses that prolonged either the reaction time (in s) or the reaction pressure (mmHg) by more than 75% in half of the animals. Ten animals were used per dose. The minimal effective dose in the shock titration test is the dose that increased the mean tolerated threshold by more than 45% in three of six post-treatment sessions. All doses refer to the free base.

Table 3 Self-administration of 33–824 in the rhesus monkey

Treatment	Time (d)	Mean daily values (24 h) Lever presses	Mean daily values (24 h) Injections	Mean daily dose (mg kg^{-1} i.v.)
NaCl (0 2 ml kg^{-1} per injection)	7	91	30	—
33–824 (0.1 mg kg^{-1} per injection + Pellet reward*)	4	183 (163†)	32 (21†)	3.2
33–824 (0.1 mg kg^{-1} per injection)	3	24	17	1.7
33–824 (0.1 mg kg^{-1} per injection)	7	40	30	3.0
33–824 (0.1 mg kg^{-1} per injection)	7	105	61	6.1
33–824 (0.1 mg kg^{-1} per injection)	7	155	99	9.9
33–824 (0.1 mg kg^{-1} per injection‡)	7	204	136	13.6
NaCl (0.2 ml kg^{-1} per injection)	1	719	391	—
NaCl (0.2 ml kg^{-1} per injection)	1	249	110	—
NaCl (0.2 ml kg^{-1} per injection)	1	192	120	—

* 15-min period daily in which the animals had simultaneous access to drug and 75 mg food pellets.

† Mean number of lever presses made and injections of drug received during 15-min food pellet access period.

‡ Naloxone (0.1 mg kg^{-1} i.v.) administered once on day 1 of this week.

and death but no catatonia, thereby confirming the results obtained in a similar study in the rat.[11] 33-824 (32 mg kg^{-1} s.c.) also caused catatonia in the rat. As in the case of β-endorphin[9,11], the catatonic effects of 33-824 were abolished by naloxone. β-Endorphin also resembles 33-824 in that it produces tolerance to its own analgesic effect[31], exhibits cross tolerance with morphine and causes physical dependence[32].

The major finding of the present studies is that by appropriate modification of enkephalin it is possible to construct a synthetic pentapeptide possessing many of the biological properties exhibited by the untriacontapeptide β-endorphin. The outstanding characteristic of 33-824 is that its long-lasting analgesic effect after systemic administration is superior to that of β-endorphin. Furthermore, 33-824 is one of the rare examples of a peptide showing pronounced biological activity after enteral administration. The ready availability and the stability of 33-824 should make it valuable for further studies of the mode of action of opioid peptides.

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Opiate analgesics inhibit substance P release from rat trigeminal nucleus

THE undecapeptide substance P (SP) may act as a transmitter released from the terminals of primary sensory nerves in the spinal cord[1–2]. SP has been shown to be highly concentrated in the terminals of small diameter fibres in the substantia gelatinosa of the dorsal horn in mammalian spinal cord, and these terminals disappear after dorsal root section[3–6]. Sensory nuclei of some of the cranial nerves, such as the spinal trigeminal nucleus, also contain a high density of SP nerve terminals[6–7]. SP is released by a calcium-dependent mechanism from the isolated rat spinal cord during electrical stimulation of the dorsal roots[8]. The peptide is also a powerful excitant of neurones in the spinal cord[2], and seems to excite selectively those cells in dorsal horn that respond to noxious stimuli[9–10]. SP may, therefore, represent the excitatory transmitter, or one of the transmitters, released from primary fibres involved in the transmission of pain. We have examined the stimulus-evoked release of SP from rat spinal trigeminal nucleus slices *in vitro* to explore the factors which may control its release from primary sensory terminals. We report here that opiate analgesics are able to suppress the stimulus-evoked release of SP. These findings may represent a mechanism for the direct spinal analgesic actions of opiates.

In the mammalian spinal cord and cranial nerve nuclei, there is a striking similarity between the anatomical distributions of opiate receptor binding sites and the endogenous opioid peptide, enkephalin[11–12]. The results of SP assays on microdissected areas of rat brain stem and spinal cord show that SP is similarly distributed (Fig. 1).

For release studies we used the techniques previously developed for studying the calcium-dependent release of SP from slices of rat hypothalamus or substantia nigra *in vitro*[13,14]. Spinal trigeminal nerve nuclei were dissected from fresh chilled 0.8 mm thick coronal sections of rat brain stem, using a stereomicroscope. The pooled tissue samples from several rats (approximately 10 mg wet weight from one rat brain) were sliced at 0.2-mm intervals in two directions with a McIlwain tissue chopper, and approximately 20 mg of tissue was transferred to each chamber of a four-channel superfusion apparatus[13]. After washing (0.375 ml min^{-1}) for 5 min with Krebs bicarbonate solution at 37 °C containing 0.5% bovine serum albumin and the peptidase inhibitor bacitracin (20 μM), superfusate samples were collected at 1-min intervals and SP-like immunoreactivity measured as described previously[13]. The spontaneous efflux of SP was equivalent to 0.2–0.4% of the total tissue content/min. As in previous experiments with slices of hypothalamus or substantia nigra[13,14], brief exposure to a medium containing 47 mM potassium chloride (K$^+$)

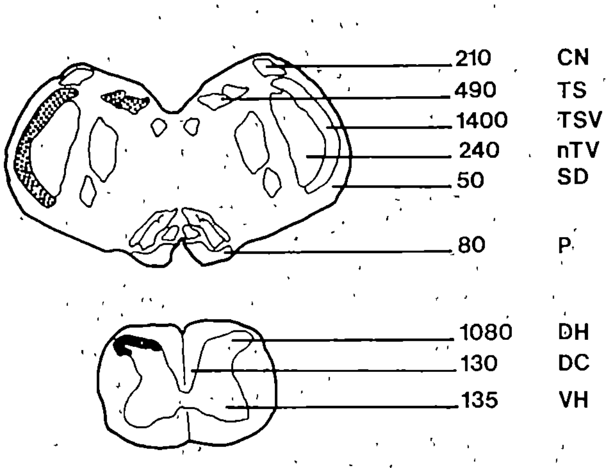


Fig. 1 Comparative distribution of opiate receptor binding and SP in rat brain stem and spinal cord. Diagrammatic representation of coronal sections of (a) rat medulla, 2 mm caudal to the obex; and (b) rat cervical spinal cord. Opiate receptor binding data are adapted from Atweh and Kuhar[11]. The left-hand side of each section shows areas with dense (solid black) or moderate (stippled) opiate receptor binding. The right-hand side of each section gives the SP concentrations expressed as pmol per g wet weight in areas obtained by microdissection. Each value is the mean ± s.e.m. of at least four animals (measured by radioimmunoassay as described previously[4]). CN, Nucleus cuneatus; TS, nucleus tractus solitarius; TSV, tractus spinalis nervi trigemini (substantia gelatinosa); nTV, nucleus tractus spinalis nervi trigemini; SD, tractus spino-cerebellaris dorsalis; P, pyramis; DH, dorsal horn; DC, dorsal column; VH, ventral horn.

elicited a marked increase in the release of immunoreactive SP, with efflux returning to basal levels soon after removal of K+ (Fig. 2*a*). Addition of morphine to the superfusing medium for 7 min before K+ application produced a concentration-dependent inhibition of the evoked release of SP, without affecting the spontaneous efflux (Fig. 2*b*). At a morphine concentration of 10 μM, the K+-evoked release of SP was almost completely supressed. Continuous superfusion with naloxone (1 μM) commencing 2 min before addition of 10 μm morphine caused an almost complete reversal of the effect of morphine on SP release (Fig. 2*c*). Superfusion with naloxone alone (1 μM) failed to affect the spontaneous efflux of SP or the K+-evoked release.

The opiate effect was stereospecific in that levorphanol (5 μM) produced an almost complete inhibition of the K+-evoked release of SP, whereas the inactive enantiomer dextrorphan did not significantly inhibit SP release at a tenfold higher concentration (50 μM) (Fig. 2*d*). Another opiate analgesic, normorphine (5 μM), similarly suppressed the K+-evoked release of SP, and this effect could be partially reversed by addition of the potent opiate antagonist diprenorphine (1 μM) 2 min before addition of normorphine (Fig. 2*e*).

A number of naturally occurring peptides with morphine-like properties, the endorphins, have been described[15-16], which vary considerably in metabolic stability and analgesic potency. In the present studies we tested the Met-enkephalin analogue [D-Ala]^2-Met-enkephalin-amide (Peninsula Laboratories), which is reported to be metabolically stable and to possess high analgesic potency[17]. A low concentration of this peptide (3 μM) almost completely suppressed the K+-evoked release of SP, and this inhibitory effect was reversed by naloxone (1 μM) (Fig. 2*f*). In other experiments (results not shown), another opioid peptide with similar metabolic stability and high analgesic potency[18], β-endorphin (Peninsula Laboratories), was found to produce 43.3 ± 8.1% (mean ± s.e.m., $n = 4$) inhibition of K+-evoked SP release at an even lower concentration (0.5 μM).

We also examined the effects of morphine and β-endorphin on the release of SP from slices of rat substantia nigra, a region of rat brain that contains a concentration of SP comparable with that found in the substantia gelatinosa of the spinal nucleus of the trigeminal nerve[4]. No significant inhibition of the spontaneous or K+-evoked release of SP from nigral tissue was observed with morphine or β-endorphin. In control experiments, exposure to K+ evoked a total increase in SP release equivalent

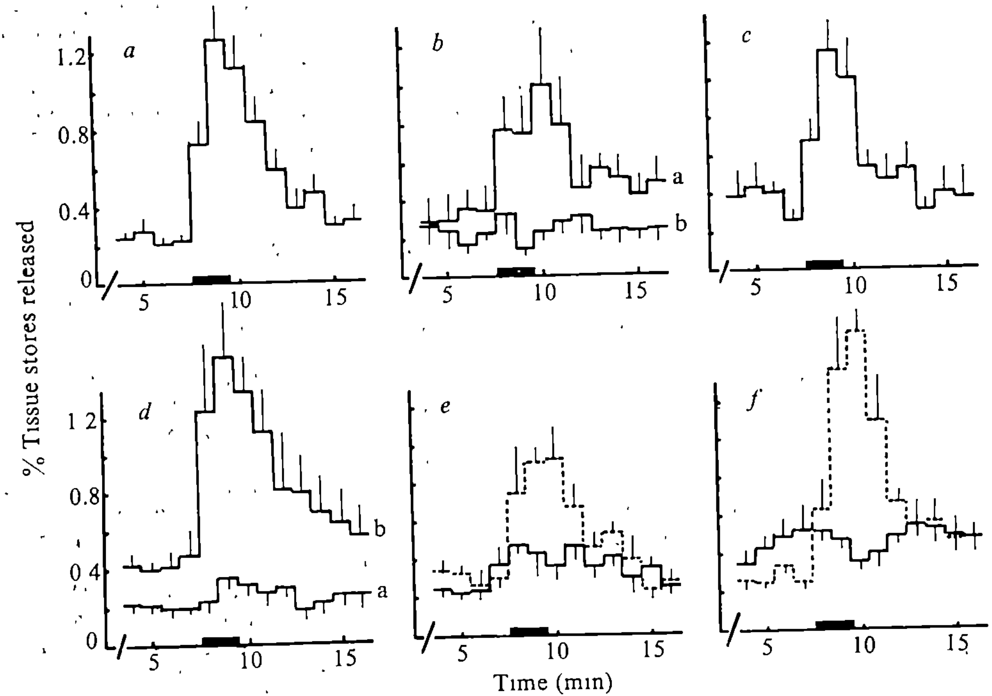


Fig. 2 Effect of opiates on the release of SP from superfused slices of spinal trigeminal nucleus from rat brain. Efflux of SP is expressed as the percentage of tissue content released per min. The spontaneous efflux of SP was approximately 30 fmol per fraction and the lower limit of sensitivity of the radioimmunoassay procedure was approximately 8 fmol. In *a–f*, each point is the mean ± s.e.m. of at least four experiments. Tissue slices were exposed to KCl-enriched Krebs solution (47 mM) for a 2-min period (indicated by horizontal black bar). *a*, Control (K+-evoked release of SP from superfused slices of spinal trigeminal nucleus); *b*, inhibition of K+-evoked release of SP by morphine (upper: 5×10^{-6} M; lower: 10^{-5} M); *c*, naloxone (10^{-6} M) reversal of morphine-induced (10^{-5} M) inhibition of K+-evoked SP release; *d*, effect of levorphanol (5×10^{-6} M : a) and dextrorphan (5×10^{-5} M : b) on K+-evoked SP release; *e*, inhibition of K+-evoked SP release by normorphine (continuous line; 5×10^{-6} M) and reversal by diprenorphine (dotted line; 10^{-6} M); *f*, inhibition of K+-evoked SP release by [D-Ala^2]-Met-enkephalin amide (3×10^{-6} M) (continuous line) and reversal by naloxone (10^{-6} M) (broken line).

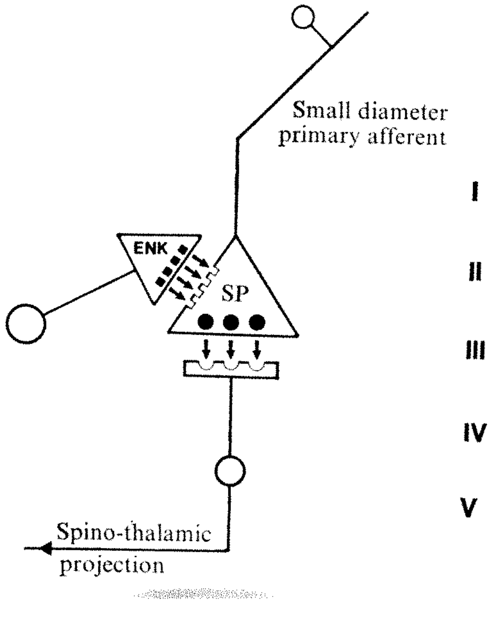
to 2.57 ± 0.26% (mean ± s.e.m.) of total tissue stores ($n = 6$). In slices exposed to 10 μM morphine, K^+-evoked release was 2.67 ± 0.29% ($n = 4$) and in two experiments with 0.5 μM β-endorphin the K^+-evoked release was 2.54 – 2.79%. Thus, opiates do not inhibit SP release from all SP-containing regions of CNS.

We believe that the present results can most easily be explained by postulating that some opiate receptors are located on primary afferent nerve terminals containing SP in the substantia gelatinosa. This would be consistent with previous work which has shown that opiates can act presynaptically to inhibit neurotransmitter release from a variety of other neurones (see, for example, ref. 19), and with the known distribution of enkephalins and opiate receptors in brain stem and spinal cord. Thus, after dorsal rhizotomy there is no change in the enkephalin content of the substantia gelatinosa area[20] but there is a significant decrease in the number of opiate receptor binding sites[21], suggesting that some of these receptors may be located on the presynaptic terminals of primary afferent neurones. Furthermore, recent immunohistochemical findings suggest that enkephalin-containing local interneurones are abundant in the substantia gelatinosa area of spinal cord and in the spinal trigeminal nerve nucleus[12,20], which represents a rostral extension of the dorsal horn, involved in the transmission of nociceptive and other information from the facial area.

The cellular mechanism by which opiates and a variety of other agents inhibit the release of transmitters from presynaptic nerve terminals remains obscure[22]. In most cases, as in the present results, control is exerted on the stimulus-evoked release of transmitter rather than on basal efflux, suggesting some interaction with the stimulus–secretion coupling mechanism, for example blockage of calcium influx[22].

A possible neuronal basis for the opiate-mediated presynaptic inhibition of primary afferent terminals and the role of enkephalins at the level of the brain stem and spinal cord is shown schematically in Fig. 3. A mechanism of this nature could account for the selective blockade by opiates of the transmission of nociceptive information in the dorsal horn[23–25], and their direct spinal analgesic actions[26]. This model could also represent the morphological substrate of the gating process proposed by Melzack and Wall[27] for the modulation of the transmission of nociceptive information within the substantia gelatinosa.

Fig. 3 Schematic representation of a possible mechanism for opiate-induced suppression of SP release. SP is shown localised within the terminal of a small diameter afferent fibre which forms an excitatory axodendritic synapse with the process of a spinal cord neurone originating in lamina IV or V and projecting rostrally. A local enkephalin-containing inhibitory interneutrone (ENK), confined to laminae II and III forms a presynaptic contact on the terminal of the primary afferent. Opiate receptor sites are depicted presynaptically on the primary afferent terminal. Numbers on the right refer to the laminae of Rexed.



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Alterations in dopamine receptor sensitivity by chronic ethanol treatment

THE hypothermic effect of an acute dose of ethanol in animals is well established[1, 2], and the development of functional tolerance to ethanol can be monitored by measuring decreased responsiveness to the hypothermia produced by ethanol[3]. Although the central neuronal systems mediating the ethanol-induced hypothermia are at present unknown, it has been found that hypothermia caused by apomorphine and piribedil in mice and rats[4, 5] is mediated by the action of these drugs on the central dopamine receptors. Since single doses of ethanol can increase the synthesis[6, 7] and/or release[8] of dopamine in brain, the possibility exists that ethanol-induced hypothermia in rodents may be in part related to activation of dopaminergic systems. If this is the case, an altered sensitivity of dopamine receptors during chronic treatment with ethanol may be responsible for the resultant decreased sensitivity to the effects of ethanol which accompanies the development of tolerance. This paper presents evidence consistent with that possibility.

Male C57B1/6 mice were fed a liquid diet containing 7% v/v ethanol or an equicaloric amount of sucrose (controls) for 7 d (ref. 3). At the conclusion of the drinking period, all mice were offered only the sucrose-containing liquid diet (withdrawal). Using this regimen, we have previously shown that tolerance to the hypothermic and behavioural effects of ethanol is clearly evident after the 7 d of drinking and continues for up to 5 d after withdrawal[3]. In contrast, withdrawal symptoms are no longer evident at 24 h after withdrawal[3]. In the present study, tolerance to the hypothermic and hypnotic effects of ethanol was determined as previously described[3] at 24 h and at 3 and 7 d after withdrawal. At these same times, the hypothermic response to intraperitoneal (i.p.) administration of a dopamine agonist, piribedil (1-(2-pyrimidyl-4-piperonyl-piperazine))[9] was assessed. To further monitor dopaminergic

Table 1 Change in body temperature of C57Bl/6 mice after drug treatment*

Drug treatment (per kg bodyweight)	Ethanol-tolerant	Control	P†
Ethanol, 3 g	1.5±0.5 (6)	3.5±0.4 (6)	<0.01
Piribedil 20 mg	1.2±0.4 (5)	2.2±0.3 (4)	<0.1
40 mg	2.0±0.1 (6)	2.9±0.1 (6)	<0.001
60 mg	2.7±0.1 (6)	3.4±0.1 (6)	<0.001
Clonidine 0.5 mg	3.9±0.4 (4)	3.4±0.5 (4)	NS

Body temperature (°C) was monitored at 15-min intervals for 30 min before i.p. injection of the drug, and at 15-min intervals for 2 h after the drug treatment. The values recorded represent the decrease in body temperature ± s.e.m. from the pre-injection baseline at 30 min after drug treatment, the time of maximum change. Rectal temperature was measured by insertion of a thermometer probe (Yellow Springs Instrument Company) 2.5 cm into the rectum. Values in parentheses represent number of animals tested.

*Ethanol-tolerant animals had been withdrawn from ethanol for 24 h.

†Values were compared by using Student's *t* test.

function, the dopamine-sensitive adenylate cyclase in brain was assayed for several days after ethanol withdrawal. Dopamine-sensitive adenylate cyclase activity was also measured in certain animals 8 h after a single i.p. injection of ethanol at 3 g per kg bodyweight. The brain section used for measurement of adenylate cyclase activity comprised the corpus striatum, diencephalon, and limbic areas[6]. This section was homogenised and assayed as described by Kebabian *et al.*[10] except that α-^{32}P-ATP was used as substrate and ^{32}P-cyclic AMP was isolated according to Saloman *et al.*[11]. The hypothermic effect of clonidine, an α-adrenergic agonist, was also monitored in both control and ethanol-tolerant animals.

Table 1 shows that animals which have been withdrawn from the ethanol-containing diet for 24 h were tolerant to the hypothermic effect of an acute dose of ethanol and were also less sensitive than controls to the hypothermic effect of piribedil. In contrast, ethanol-tolerant and control animals were equally sensitive to the hypothermic effects of the α-adrenergic stimulant clonidine. The change in the dose–response pattern of the hypothermic effects of piribedil in the ethanol-tolerant animals suggested a decrease in central dopaminergic receptor responsiveness to this agonist.

The dopamine-sensitive adenylate cyclase has been reported in most instances[12] to have many of the characteristics of the dopamine receptor *in vivo*[10, 13]. At 8 h after injection of a single dose of 3 g per kg ethanol, we found that basal adenylate cyclase activity and the stimulation of the enzyme by dopamine was similar in brains of ethanol-treated and control animals (that is at 10^{-5} M dopamine, the percentage stimulation over basal values was 66.8±9.1 (s.e.m.) in ethanol-treated and 66.4±3.9 in control animals). On the other hand, Fig. 1 illustrates the response of the dopamine-sensitive adenylate cyclase of mouse brain to various doses of dopamine in control animals and animals fed the ethanol-containing diet for 7 d and withdrawn for 24 h. The adenylate cyclase from the ethanol-tolerant animals showed a decreased response at all dopamine concentrations tested, and the response did not seem to reach the same maximum value as seen in control animals. Basal values were the same in control and ethanol-tolerant animals (see legend to Fig. 1).

The data in Table 2 demonstrate that the decreased sensitivity to the hypothermic effect of both ethanol and piribedil was still present at three days after ethanol withdrawal, but was no longer evident by 7 d after withdrawal. Similarly, dopamine-sensitive adenylate cyclase from ethanol-tolerant animals was significantly less sensitive to dopamine stimulation at three, but not at 7 d after withdrawal (see Table 2).

Seeber and Kuschinsky[14] have demonstrated that the dopamine-sensitive adenylate cyclase in brains of rats withdrawn from chronic barbiturate treatment was less responsive to dopamine stimulation than enzyme from control animals. These authors,[14] however, found little difference in response

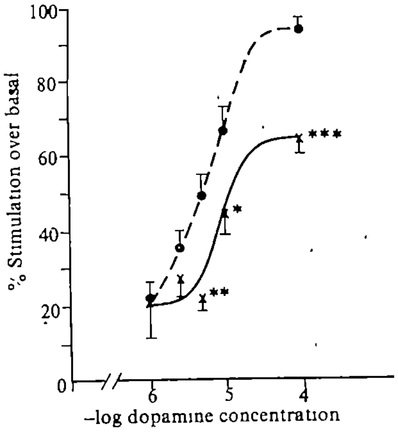

Fig. 1 Stimulation of dopamine-sensitive adenylate cyclase in brain of control (●) and ethanol-tolerant (×) animals. Animals were killed 24 h after withdrawal from the ethanol-containing diet, after 7 d of consumption. Brains were dissected and dopamine-sensitive adenylate cyclase activity was measured as described in the text. Basal values for control and ethanol-tolerant animals were not significantly different (respectively, 31.5±3.5 and 29.9±3.1 pmol cyclic AMP produced per 3 min). Dopamine concentration is molar, and results are expressed as per cent stimulation over basal values ± s.e.m. Values which are significantly different in ethanol-tolerant and control animals are indicated as follows: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ (*t* test).

to dopamine between ethanol-treated and control animals, but their animals were not shown to have developed any tolerance to ethanol, and the development of central nervous system tolerance may be critical for the observed alterations in adenylate cyclase activity.

Our finding that the maximal response of the adenylate cyclase to dopamine is decreased in ethanol-tolerant animals, while the concentration of dopamine producing a half-maximal response seems similar in both control and ethanol-treated animals, is in line with previous suggestions that changes in receptor sensitivity are related to alterations in receptor density rather than affinity for agonist[15,16].

We have recently demonstrated that partial destruction of brain noradrenergic systems by 6-hydroxydopamine (6-OHDA) before ethanol consumption blocked the development of tolerance to ethanol[17], but the same treatment administered

Table 2 Time course for the disappearance of ethanol tolerance and of alterations in dopamine receptor sensitivity

Drug	Time after withdrawal (d)	Change in body temperature Ethanol-tolerant	Control	P*
EtOH 3 g per kg	3	1.8±0.2 (6)	3.5±0.2 (6)	<0.05
	7	3.9±0.2 (6)	3.3±0.3 (6)	NS
Piribedil	3	0.5±0.2 (3)	1.8±0.6 (3)	<0.1
	7	1.8±0.2 (8)	2.0±0.2 (8)	NS

	Time after with-drawal (d)	Adenylate cyclase activity, % stimulation Ethanol-tolerant	Control	Δ	P
Dopamine, 5×10^{-6} M	3	41.7±3.5 (9)	55.0±4.8 (9)	13.3	<0.05
	7	49.1±5.1 (6)	46.6±4.1 (6)	2.5	NS

Ethanol and piribedil were administered i.p. and body temperature monitored as described in the text and legend to Table 1. Dopamine-sensitive adenylate cyclase was measured as described in the text; basal values were similar to those in the legend of Fig. 1 and were not significantly different between ethanol-treated and control animals. All values represent mean ± s.e.m.; values in parentheses represent number of animals or, for adenylate cyclase activity, number of experiments.

*Values on any particular day were compared by the Student's *t* test.

after tolerance had developed did not prevent expression of tolerance[18]. Our previous data thus indicated that neurones other than those of the noradrenergic system would be responsible for the expression of tolerance to the hypothermic effects of ethanol. The postulated interaction between noradrenaline and dopamine-containing neurones[19], in which activity in one system could exert a modulatory influence on the other system, would lend support to a hypothesis that dopaminergic systems may be involved in expression of tolerance to the hypothermia produced by ethanol.

Although the time course of disappearance of decreased dopaminergic sensitivity seems to parallel the time course for disappearance of tolerance to ethanol, future studies should also evaluate the pattern of development of the alterations in dopamine receptor sensitivity, in order to more clearly define the possible relationship of such changes in the dopaminergic systems to the development of physical dependence on ethanol.

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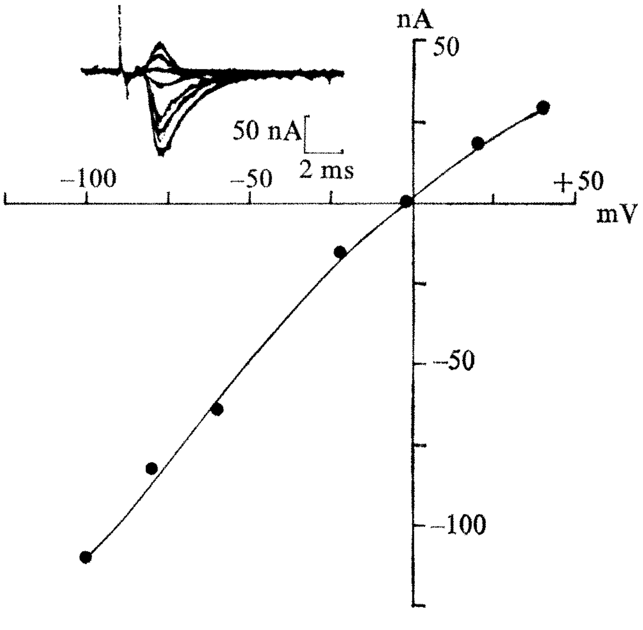
Desensitisation does not selectively alter sodium channels

WHEN depolarising drugs are applied to the endplate region of skeletal muscle fibres, the postjunctional membrane undergoes a rapid increase in ionic conductance. This increased permeability, largely for Na^+ and K^+, is slowly reversed if the agonist application is sustained. The gradual diminuition of response to agonist is due to endplate desensitisation[1]. Many factors influencing desensitisation have been described, but the molecular mechanisms responsible for the development of desensitisation remain unknown. Two fundamental types of mechanisms have been postulated to explain the phenomenon of desensitisation[2-6]. In the first, desensitisation is viewed as resulting from an alteration in the chemical receptor or binding site. The second type of mechanism suggests that desensitisation results from an alteration at some extrareceptor site such as that controlling the ionic channel in the endplate membrane. Kuba and Koketsu[7] have shown that the reversal potential for acetylcholine was shifted to more negative values at desensitised endplates. They argued that this change was due to a selective decrease in G_{Na} and therefore supported the view that an extrareceptor mechanism was important in the development of desensitisation. In contrast Koester[8] found no significant alteration in the reversal potential at desensitised endplates. In view of these conflicting observations we have re-investigated the question of whether there is or is not a selective alteration in endplate conductance during desensitisation. We report here that desensitisation does not selectively alter sodium channels.

All experiments were performed *in vitro* on sciatic nerve–sartorius muscle preparations from the frog, *Rana pipiens* maintained in a phosphate-buffered Ringers solution[6] or a Tris-buffered, Na^+-free, isotonic K^+ propionate solution[9]. For some experiments, the phosphate-buffered Ringers solution was made 2.3 times hypertonic by the addition of sucrose to minimise muscle contraction during carbachol application[6,10]. In other experiments, neuromuscular transmission was depressed by the addition of curare (2–3 mg l^{-1}) to the bathing solution to reduce the amplitude of the postjunctional conductance change. Endplate desensitisation was induced with carbachol. The carbachol was either added directly to the solution bathing the muscle preparation or was locally microperfused on to the endplate region of individual muscle fibres by hydrostatic pressure from an 85–100 μm diameter pipette placed within 50 μm of the recording and current-passing electrodes[6,10]. Junctional regions of individual fibres were located visually by following nerve twigs to the last node of Ranvier using transmitted light and a magnification of 300. Conventional electrophysiological techniques were used to measure resting membrane potentials. The neurally-induced endplate current (EPC) and carbachol-induced currents were determined with point voltage-clamp techniques using a two microelectrode voltage clamp similar in principle to that of Takeuchi and Takeuchi[11] and in detail to that described by Gibbons and Fozzard[12].

The possibility that desensitisation results in a selective decrease in Na^+ conductance at the endplate membrane was evaluated by comparing the reversal potential for neurally-evoked EPCs of curarised preparations to those of partially-desensitised endplates. We reasoned that a selective decline in Na^+ conductance during desensitisation would shift the reversal potential to a more negative value. Curare was used so that non-desensitised EPCs had similar amplitudes to desensitised non-curarised EPCs. Individual fibres were voltage-clamped over the range −100 mV to +40 mV, and the reversal potentials were determined by interpolation (Fig. 1). The reversal potential obtained from curarised (2–3 mg l^{-1}) junctions ($E_r = -2.4 \pm 2.8$

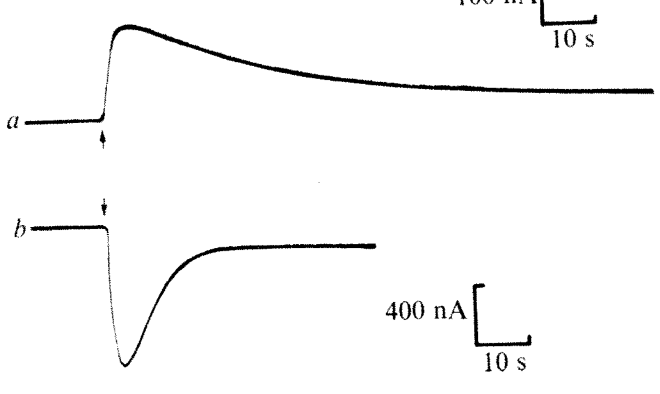
Fig. 1 Example of the current–voltage relationship obtained at a desensitised endplate. The muscle from which this record was obtained was bathed in a phosphate-buffered Ringers solution containing 55 μM carbachol. The data were gathered after exposing the muscle to carbachol for 25 min. In this particular fibre the resting potential was −44 mV. The inset shows the current traces from which the current–voltage curve was plotted. An upward deflection indicates outward current and a downward deflection indicates inward current. The EPC reversal potential, determined by interpolation is −2 mV.



mV; mean ± s.e., $n = 9$) was similar to that obtained from 55-µm carbachol-desensitised junctions ($E_r = -4.4 \pm 1.2$ mV; mean ± s.e., $n = 14$). This difference was not statistically significant ($P > 0.05$, Student's t test). These values of the reversal potential are comparable to those reported by others[13,14]. Estimates of the reversal potential were obtained during a 20–110-min exposure to curare or a 10–80-min exposure to 55 µm carbachol. In some carbachol-treated preparations there was a progressive decrease in the neurally-evoked EPC amplitude during this period whereas in other preparations the amplitude remained relatively constant during drug exposure. Nevertheless, the value of the reversal potential was similar in both instances. In the carbachol-treated fibres, the resting potential was initially low and progressively returned towards the pre-carbachol level with time[6]. This was taken as evidence for the continued development of desensitisation in these fibres.

If desensitisation selectively affects G_{Na} without influencing G_K, then carbachol-induced currents should not decay at E_{Na} where the EPC is primarily a potassium current. To test this possibility, the activation–desensitisation sequence was evaluated at E_K and E_{Na}. This was accomplished by monitoring the EPC produced by local microperfusion of 110 µM carbachol on to individual end plates voltage clamped at −100 mV or +50 mV, two values assumed to be reasonable approximations of E_K and E_{Na}, respectively. To minimise muscle contraction, these preparations were maintained in a hypertonic phosphate-buffered Ringers solution[10]. Microperfusion of 110 µM carbachol on to the endplate region of these voltage-clamped fibres induced a transient current superimposed on the holding current. At both voltages, the carbachol-induced EPC developed with time to a peak of a few seconds, then slowly decayed toward the pre-carbachol level even though the carbachol application was continued (Fig. 2). The decay of the carbachol-induced EPC indicates that desensitisation occurred at both E_K and E_{Na}. We have used the time constant of current decline as an index of the time course of desensitisation. In these two examples, desensitisation developed more slowly in the fibre voltage-clamped at +50 mV ($\tau = 38.6$ s) than in the other fibre voltage-clamped at −100 mV ($\tau = 7.0$ s). Similar results were obtained from three other muscle preparations, one fibre from each preparation being voltage-clamped at −100 mV and another at +50 mV during carbachol application. We attribute the difference in desensitisation rates at these two voltages to the voltage dependence of desensitisation as described by Magazanik and Vyskocil[4]; hyperpolarisation accelerating and depolarisation slowing the process.

Fig. 2 Time course of endplate current produced by sustained microperfusion of 110 µM carbachol. *a*, Response from an endplate voltage-clamped at +50 mV. *b*, Response from an endplate voltage-clamped at −100 mV. −100 mV and +50 mV were assumed to be reasonable approximations of E_K and E_{Na}, respectively. To minimise muscle contraction during carbachol microperfusion, these muscles were bathed in a phosphate-buffered Ringers solution made 2.3 times hypertonic by the addition of sucrose. Arrows indicate the onset of carbachol microperfusion. An upward deflection indicates outward current and a downward deflection indicates inward current.



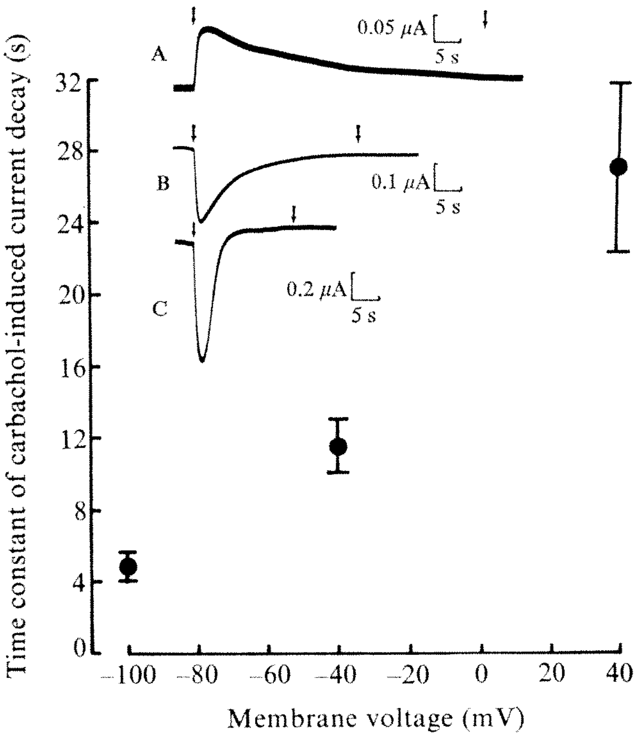


Fig. 3 Voltage dependence of desensitisation produced by 250 µM carbachol in K^+ depolarised muscle fibres. These data were obtained from fibres bathed in a Tris-buffered, Na^+-free, isotonic K^+ propionate solution. The time constant of carbachol-induced current decay during continued carbachol perfusion is plotted as a function of membrane potential. Each point represents the mean ± s.e. obtained from at least five fibres. The inset shows examples of 250 µm carbachol-induced currents from three different fibres measured in voltage clamp conditions. The fibres were voltage-clamped to: +40 mV (*a*), −40 mV (*b*), and −100 mV (*c*), respectively. Arrows indicate the onset and termination of carbachol application. An upward deflection indicates outward current and a downward deflection indicates inward current.

Desensitisation occurs in K^+-depolarised muscle preparations in the absence of extracellular sodium[9,10]. In these conditions, the agonist-induced current is carried primarily by K^+. We therefore evaluated desensitisation induced by local microperfusion of 250 µM carbachol in voltage-clamped muscle fibres (V_C at either −100 mV, −40 mV, or +40 mV) maintained in a Na^+-free, isotonic potassium propionate Ringers solution. Figure 3 indicates that the rate of desensitisation in these potassium depolarised fibres is voltage dependent, and the dependence is similar to that observed in polarised fibres (compare with Fig. 2). These observations indicate that the voltage dependency of desensitisation is maintained regardless of the ionic current-carrying species.

In agreement with Koester[8], we find no difference in EPC reversal potential between curarised and partially desensitised endplates. The data presented here suggest there is no selective alteration in G_{Na} at desensitised endplates. Because the current–voltage relationship for the EPC is nonlinear[13,14], estimates of the reversal potential obtained by extrapolation can be misleading. Although Kuba and Koketsu's[7] plots show little evidence of nonlinearity, this may account, in part, for the conflict between conclusions drawn previously by Kuba and Koketsu[7] and with those of Koester[8] and the present report. It should be noted that the experimental conditions used in this study and by Koester[8] are substantially different from those of Kuba and Koketsu[7]. In the former desensitisation was induced by applying carbachol to the bathing solution whereas in the latter desensitisation was produced by iontophoretic application of acetylcholine. Consequently, the different results may be due to different experimental conditions. But, in agreement with the data presented here, Manalis and Werman[15], using experimental conditions similar to those of Kuba and Koketsu[7], have demonstrated by interpolation that desensitisation does not alter the acetylcholine reversal potential. In summary, our results show that desensitisation develops whether the endplate current is carried mainly by Na+ and K+ or by Na+ and K+ individually, and that differences in time course of desensitisation apparently are due to the voltage dependence of the desensitisation process[4].

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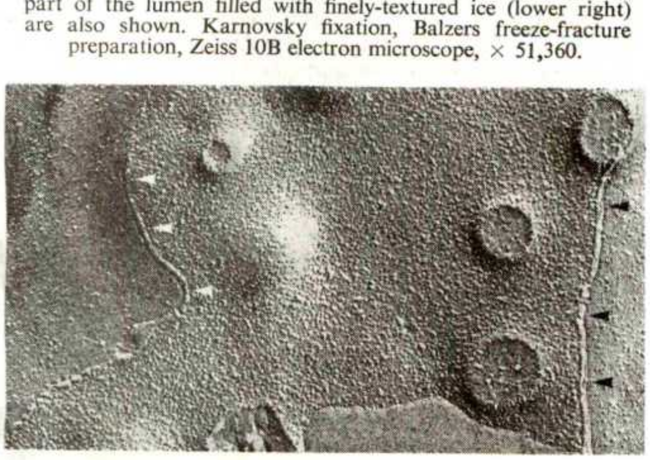
Significance of extensive 'leaky' cell junctions in the avian salt gland

Current models for hypertonic sodium secretion by salt glands assume there are physiologically tight junctions between the principal cells of the secretory tubules and that these cells actively secrete sodium across the cell membrane at the lumen in a one-stage process[1]. Our recent data require consideration of a new two-stage hypothesis concerning the secretory mechanism. Our freeze–fracture studies demonstrate that these cell junctions (*zonulae occludentes*) in the nasal salt gland of the herring gull (Fig. 1) should be classified as 'very leaky'[2]. Also, the extraordinary conformation of the apices of the principal cells within the epithelium makes these cell junctions most extensive (Fig. 2). Therefore, we propose that the salt glands of vertebrates produce their hypertonic effluent from the primary isotonic secretion of the peripheral cells at the ends of the secretory tubules, followed by the secondary reabsorption of water through the leaky junctions of the principal cells and not simply by the extrusion of Na+ across the luminal membranes of the principal cells. This new hypothesis is consistent with the accepted theories of sweat gland and salivary gland function, except that in the salt gland the second stage after isotonic secretion is water absorption, rather than salt reabsorption.

Fig. 1 Freeze–fracture image through the luminal membranes (*P*-faces) of three adjacent apical processes of the principal cells in the salt gland of a herring gull. It demonstrates clearly that the *zonulae occludentes* (arrowheads) are single-stranded. Circular profiles of the bases of several microvilli and a small part of the lumen filled with finely-textured ice (lower right) are also shown. Karnovsky fixation, Balzers freeze-fracture preparation, Zeiss 10B electron microscope, × 51,360.



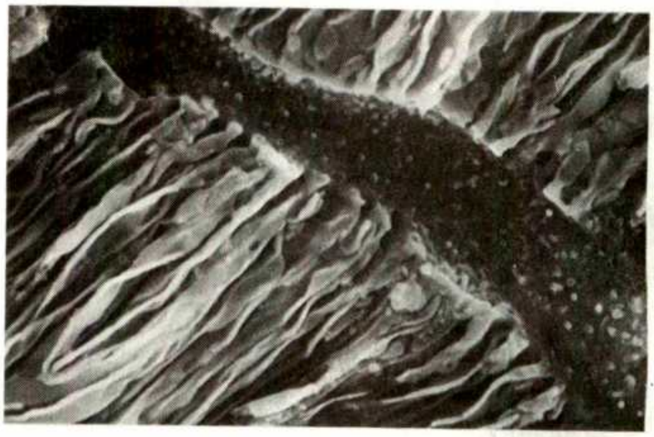


Fig. 2 The lumen of a secretory tubule from the nasal salt gland of a herring gull traverses diagonally from upper left to lower right through this scanning electron micrograph. Knoblike microvilli dot the surface of the lumen. The intermeshing apical processes of the principal cells form the wall of the lumen. Folded lateral surfaces of the principal cells (lower left and upper right) delimit long intercellular channels extending away from the luminal cell junctions. The complementary surfaces of overlying principal cells were pulled purposely out of their positions during specimen preparation. Karnovsky fixation, osmium-thiocarbohydrazide coating, critical point drying with CO_2, recorded with an AMR-1000 scanning electron microscope, × 5,928.

Within the salt gland the lobules contain branched, blind-ended secretory tubules (Fig. 3) that radiate from a central excretory canal[3]. The diameter of the lobules and the length of the secretory tubules are both directly correlated with the concentration of Na+ in the effluent of the gland[4]. Also, the size and height of the principal cells that predominate in these tubules increase progressively along a tubule from the periphery toward the centre of each lobule[5]. Within the cytoplasm of these cells large, abundant mitochondria are closely associated with the lateral membranes along the intercellular channels[6]. In addition, cytochemical[7–10] and autoradiographic[11] studies show that the enzyme system responsible for sodium transport is found in these lateral plasma membranes and not in the luminal membranes of these cells.

In contrast, the cuboidal peripheral cells (Fig. 3), situated in the blind end of each tubule are relatively small. They resemble closely the 'clear' secretory cells that are found in the secretory tubules of eccrine sweat glands, and have cytochemical[12,13] and ultrastructural[6] characteristics that are quite different from the principal cells.

Freeze–fracture images (Fig. 1) through the plasma membranes at the luminal interface of principal cells show that the *zonulae occludentes* consist of a single strand. Thus, by comparison with other epithelia[2], this junction must be classified as very leaky. This classification is substantiated also by earlier tracer studies on salt glands[14,15].

The strikingly complex plicated lateral surfaces of the principal cells in the secretory tubules of the salt gland of the herring gull is revealed by the scanning electron microscope (Fig. 2). Folds of these membranes form the boundaries of long intercellular channels that extend from the apical cell junctions to the extracellular space at the base of the epithelium. In addition, the complicated intermeshing at the lumen of the apical ends of the principal cells[6], means that the leaky cell junctions are unusually extensive.

Figure 3 shows our conception of how a secretory tubule

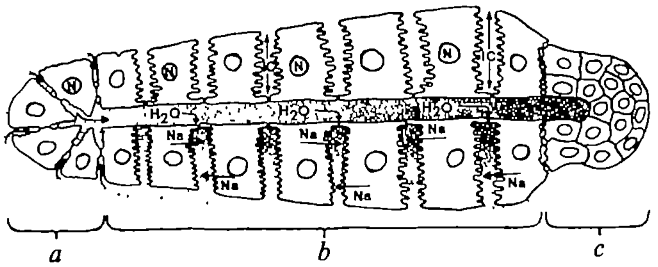


Fig. 3 In this diagram of a single secretory tubule in a salt gland, the number of cell junctions as well as the complexity of the intercellular channels are greatly reduced, and the diameter of the lumen of the tubule is much enlarged. The terminal segment (*a*) of the tubule is composed of small peripheral cells; the remainder of the tubule (*b*) is lined by principal cells. The central excretory canal (*c*) is continuous with an excretory duct (not shown). The intercellular channels (IC) are closed at their luminal ends by leaky cell junctions. The density of the stipple in the lumen and in the intercellular channels indicates the concentration of Na$^+$.

produces a hypertonic secretion. Isotonic fluid secreted by the peripheral cells at the terminal cul-de-sacs (*a*) flows along the lumen of the tubule towards the central canal (*c*). As this secretion moves over the apices of the principal cells that line most of the tubule (*b*), water is absorbed from this fluid through the leaky junctions between the principal cells and into the intercellular channels where a standing gradient[16] of Na$^+$ is maintained by the sodium pumps in the lateral membranes of the cells. Na$^+$ ions are most concentrated at those ends of the channels near the leaky cell junctions that border the lumen of the tubule and are least concentrated at the bases of the channels where they are open to the subjacent extracellular space. In maintaining the standing gradient, NaCl is recycled by the principal cells. NaCl enters the cells from the blood across the basal membrane, is pumped out the lateral membrane into the intercellular channels, and then returns to the blood. The NaCl moves from the blood into the cell, passing down a concentration gradient created by the lateral extrusion pumps. As a consequence of this standing gradient, water flows from the lumen into the intercellular channels. The concentration of Na$^+$ in the fluid that remains in the lumen of the tubule will approach the maximum of the standing gradient that is achieved in the intercellular channels. Accordingly, the height of the principal cells, and hence the length of the intercellular channels, along with the level of operation of the Na$^+$ pumps, produce also a standing gradient within the lumen of the tubule and affect the Na$^+$ concentration of the final effluent. Simply stated, the principal cells do not secrete the hypertonic effluent of the glands. Instead, water is passively reabsorbed through the leaky cell junctions from the isosmotic fluid that is secreted by the peripheral cells. This differs from the original form of the standing gradient hypothesis[16] which postulated water flow across the apical and lateral cell membranes.

This hypothesis requires that NaCl extruded laterally into the intercellular spaces is recycled NaCl, and does not come from the lumen, also that the plasma membranes of the principal cells must be relatively impermeable to water. This latter requirement is not unique, since cells with similar properties are found, for example, within the loop of Henlé in the mammalian kidney. It is also consistent with experimental data on intercellular osmolarity in salt glands[17,18]. In addition, extracellular coats that are detectable at the surface of the principal cells[14, 19] might also affect the permeability of the membrane.

Our hypothesis solves the dilemma of the orientation of the sodium pumps. Localisation of the sodium pumps along the basal and lateral membranes[7–11] required under earlier hypotheses[1] that Na$^+$ be pumped into the cells, an unprecedented orientation. In this new hypothesis, the sodium pumps have their usual outwards orientation and still have a role in hypertonic secretion.

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Composition of sarcoplasmic reticulum *in situ* by electron probe X-ray microanalysis

EXCITATION–contraction (E–C) coupling in striated muscle[1,2] involves depolarisation of the surface membrane that propagates through invaginations of the surface membrane, the transverse tubular (T) system, to the interior of the fibre. Stored Ca is then released from the adjacent sarcoplasmic reticulum (SR), and activates the contractile proteins. The mechanism of release of Ca from the SR is not definitely known, although it is thought to be triggered by the T-tubule action potential either through electrical coupling, possibly involving voltage dependent charge movement[3–5] across the T-SR foot processes[6–7] and depolarisation of the SR[2,8,9] and/or a chemical coupling involving a Ca induced Ca release[9–12]. Depolarisation of the sarcoplasmic reticulum in skinned muscle fibres by Cl induces Ca release[2]. The evaluation of the various models of E–C coupling requires some knowledge of whether the ionic distribution across the SR membrane can give rise to a potential and, specifically, whether the content of the SR resembles that of the extracellular or of the intracellular fluid. So, to measure the ionic composition of individual elements of the sarcoplasmic reticulum *in situ*, we used electron probe X-ray microanalysis of frozen–dried thin section of a fast striated muscle The data we report here show that neither Cl nor Na are compartmentalised in the SR, and that their concentrations in the SR (on a dry weight basis) do not differ from those in the surrounding cytoplasm. Ca was found to be sequestered in the sarcoplasmic reticulum confirming other studies (refs 13, 14 and see refs 1, 2 for reviews). These findings (albeit on a dry weight basis) do not support the existence of a Cl or Na potential across the SR membrane in resting muscle.

The swimbladder muscle of the toadfish *Opsanus tau* is a very fast twitch muscle with a time to peak contraction of 5–8 ms and an extraordinary development of the sarcoplasmic reticulum[15]. The extent of the SR (approximately 30% of fibre volume, C. Franzini-Armstrong, personal communication) and its very regular arrangement of two triads per A band (Fig. 1) make it very suitable for viewing in unfixed frozen-dried thin sections. The muscles were shot into supercooled Freon 22, sectioned at −130 °C and

Table 1 Elemental concentrations of sarcoplasmic reticulum and cytoplasm of toadfish swimbladder determined by paired 'small spot' electron probe analysis (mmol per kg dry weight)

Region analysed	Na	P	S	Cl	K	Ca	Mg
Cytoplasm (between SR)	30±4.3	275±2.0	335±2.0	47±1.1	510±2.7	4±0.8	47±1.9
Sarcoplasmic reticulum	34±4.2	443±2.5	362±2.0	42±1.0	589±2.8	77±0.9	40±1.8

Values are means ± s.e.m., $n = 25$ pairs. We used a Philips EM 301 transmission electron microscope with a goniometer stage modified to accept a 30-mm^2 (20° specimen to detector angle, 0.067 sr solid angle, 160 eV resolution) Kevex Si(Li) X-ray detector interfaced with a Kevex 5100 multichannel analyser and a Tracor Norther NS 880 computer and TN 1,000 magnetic tape recorder. A modified Philips liquid nitrogen cooled holder, operated at −100 to −110 °C, was used to minimise contamination and mass loss. The detailed characteristics of the system have been described previously[19]. Some analyses were done with the Philips EM 400 operated with an LaB$_6$ source and interfaced as above. The better vacuum (2×10^{-7} torr) allowed us to obtain longer counts without contamination and the higher brightness of the LaB$_6$ source yielded a higher count rate. The probe diameters were 50–100 nm. The SR was probed in regions adjacent to the T-tubules where these could be identified. The regions of the cytoplasm analysed were within 200 nm of their respective SR pairs. The measurement of elemental concentrations is based on the fact, pointed out by Hall[18], that for elements of atomic number $Z \geq 11$–20 in concentrations of less than 1 mol per kg, the characteristic peak/X-ray continuum ratio is linearly related to the concentration/dry mass. The peak counts in the unknown peak and its error of measurement are computed together with the counts in the continuum band 1.34–1.64 keV. The basic equation for quantitation is $C_x = (I_x/I_b)\chi\, W_x$, where C_x is the concentration of element χ, I_x is the number of characteristic peak counts for that element, I_b is the number of continuum counts and W_x is a quantitation parameter relating concentration to peak/background ratio and is obtained from standards. W_x includes the effects of ionisation cross sections, fluorescence yield and detector efficiency for each specific element.

The X-ray spectrum obtained in a transmission electron optical column from a thin section includes the characteristic peaks due to elements $Z \geq 11$ present in the specimen and the associated continuum largely arising from the organic matrix. It also contains instrumental peaks (the largest one being the Cu signal $K_\alpha = 8.047$ keV) from the specimen grid and holder, with an associated extraneous continuum, and low energy noise probably due to electrons reaching the detector. These extraneous signals and the associated continuum can be measured by collecting the spectrum generated with the beam passing through an empty grid hole, and are subtracted by the computer routine as is the low energy noise[19].

freeze–dried as reported previously[16]. Frozen–dried thin sections of unfixed muscle are shown in Fig. 2 and can be compared with a conventionally glutaraldehyde-osmium fixed section of the same muscle shown in Fig. 1. The junctional gap between the T-tubule and terminal cisterna[6,7] is also present in the unfixed frozen sections, but the T-tubule is not as flattened as in fixed material (Fig. 2*b*). The longitudinal SR is narrower in the cyro sections and has a laminated appearance, suggesting that measurements in conventionally fixed material may give rise to overestimates of the *in vivo* SR volume. A collapsed longitudinal SR has been previously observed in frog striated muscles fixed in the presence of polyvalent cations[17] and in freeze–fracture replicas of unfixed muscle (Franzini-Armstrong, Reese and Heuser, personal communication).

The concentrations of Na, P, S, Cl, K, Ca and Mg in, respectively, the SR or the adjacent cytoplasm (25 paired analyses with 50–100 nm probes) are shown in Table 1. Because the quantitation is based on the ratio of the characteristic peak to the X-ray continuum counts[18,19] from frozen–dried material, the data are expressed as mmol per kg dry weight. The mean Ca concentration in the SR of resting muscle is 77 ± 0.9 (s.e.m.) mmol per

Fig. 1 Longitudinal section from a toadfish swimbladder muscle fixed in glutaraldehyde, post fixed in osmium and embedded. T-tubules indicated by arrows. (× 41,580).

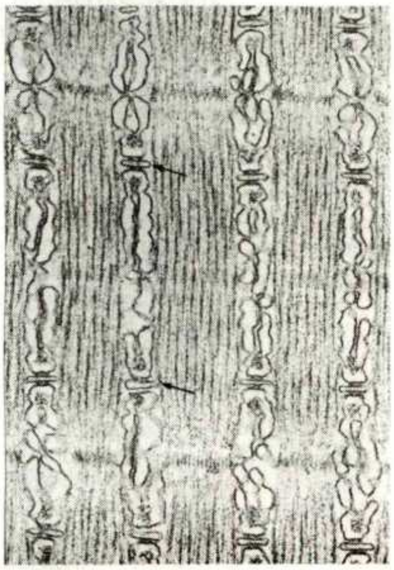
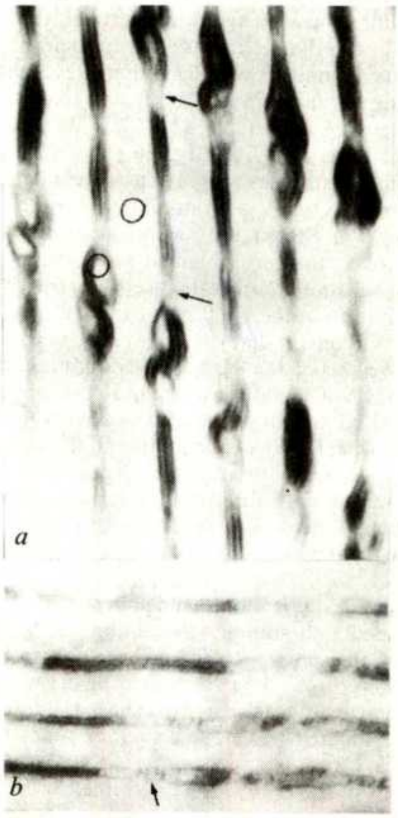


Fig. 2 Longitudinal cryo sections from toadfish swimbladder muscle. The muscle mounted on a low mass stainless steel mesh holder was frozen by shooting it into supercooled (−164 ± 2 °C) Freon 22 and sectioned at −130 °C in a modified LKB cryokit. The sections were picked up dry onto Cu grids with carbon foils, dried at < 10^{-6} torr overnight, carbon coated and stored dessicated. This section was stained with osmium vapour under vacuum to enhance contrast. Electron probe X-ray microanalyses were generally done on similar but unstained material. A detailed description of the freezing and cryoultramicrotomy is given elsewhere[16]. T-tubules are indicated by arrows. Circles indicate the approximate probe size used for the analyses summarised in Table 1. *a*, × 39,960. *b*, × 53,680.



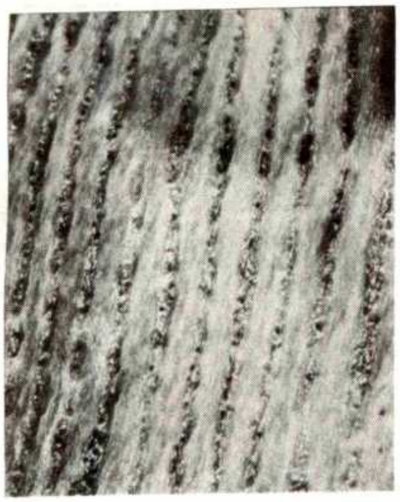


Fig. 3 Longitudinal cryo section from a damaged toadfish swimbladder muscle fibre. Unstained. The sarcoplasmic reticulum is loaded with electron dense granules. (× 4,650.)

kg. Because much of the phosphorus in the SR region (Table 1) could be within membrane phospholipids, these experiments do not indicate whether P (phosphate) is a counterion for Ca. The Ca concentration in the regions of cytoplasm between the SR shown in Table 1 was higher than in frog muscle[16]. This may reflect some overlap of obliquely oriented SR in the narrow segments of cytoplasm analysed (Fig. 2) and/or Ca binding to the parvalbumins in fish muscle[20]. Recent studies, however, indicate that the parvalbumin contents of frog and fish muscle are similar[24].

The Na and the Cl concentrations in, respectively, the SR and adjacent cytoplasm were not significantly different. This finding does not support the existence of a Na or Cl potential across the SR membrane, however, electron probe analysis of freeze–dried cryo sections measures total (bound and free) ion contents rather than activities. The greater (by approximately 80 mmol per kg) concentration of K detected over the SR than over the cytoplasm is similar to our observations on frog stiated muscle SR[16] and could arise from differences in hydration between the two regions or from K binding to the SR and its acidic proteins[21,22].

The results of analyses of 1–6 μm diameter areas that included both SR and cytoplasm (in 13 fibres from 4 toadfish) were: (mmol per kg dry weight ± s.e.m.): Na, 42 ± 7.2; P, 360 ± 4.3; Cl, 56 ± 1.9; K, 539 ± 5.1; Ca, 26 ± 1.6; Mg, 36 ± 3.0. The relatively high (26 ± 1.6 mmol per kg dry weight) Ca content detectable with the probe parameters used is primarily due to the large volume occupied by the SR in the swimbladder muscle. The other elemental concentrations obtained with these large diameter probes also substantiate the absence of 'extracellular' ion compartmentalisation in the SR (apart from Ca), shown in Table 1. The bulk NaCl content that would have been expected (277 mmol per kg dry weight) on the basis of an SR containing extracellular fluid (231 mM NaCl) and constituting 30% of fibre volume is very much higher than the measured values (Na, 42 ± 7.2; Cl, 56 ± 1.9 mmol per kg dry weight). Minor differences between these Na and Cl concentrations and those measured with small probes (Table 1) were due to fibre-to-fibre variations. The Na and Cl concentrations measured with large (1–6 μm) or small (50–100 nm) probes in the same fibre were not significantly different.

In one damaged fibre not included in the tabulated results, the SR was loaded with granules containing Ca, Mg and P (Fig. 3). Analyses of this fibre with large diameter probes showed Ca concentrations of 76 ± 3.5 m mmol per kg dry weight indicating a threefold increase in total fibre Ca. The maximum Ca capacity of the SR is estimated to be three to four times greater than its physiological Ca content[2,23].

The results of this study are in agreement with our findings on frog striated muscle[16], that the Cl concentration of the SR does not approach extracellular levels. In addition, the results on toadfish SR show a similar absence of compartmentalised Na; this excludes the possibility that the relatively low Cl concentration in the SR is due to Donnan exclusion by acidic proteins. We conclude that the T–SR junction in resting muscle is not freely permeable to Na or Cl.

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Myoblast myosin phosphorylation is a prerequisite for actin-activation

CYTOPLASMIC myosins are found in most non-muscle cellular systems studied to date[1]. In vertebrates these myosins are composed of two heavy chains of 200,000 molecular weight (MW) and four light chains, two each of 20,000 and 15,000 MW. A number of cytoplasmic myosins, as well as skeletal, smooth, and cardiac muscle myosin, can be enzymatically phosphorylated[2–6]. That is, those light chains of MWs 18,000–20,000, the so-called P-light chains[6], can undergo phosphorylation catalysed by a myosin light chain kinase[7,8]. In this reaction the terminal phosphate of ATP is transferred to a specific amino acid residue on the myosin P-light chain, up to one mole of phosphate per mole of light chain[9,10]. A myosin light chain phosphatase that catalyses the removal of the covalently-linked phosphate group from the P-light chain, returns the myosin to the non-phosphorylated state[5,11]. Phosphorylation of human platelet myosin[4], guinea pig vas deferens[3] and chicken gizzard[12,13] smooth muscle myosin results in an increase in the actin-activated ATPase activities of these myosins. Although the phosphorylation of the P-light chain of rabbit skeletal muscle myosin has been described[10], no functional change in this myosin's activity due to phosphorylation has been demonstrated[11]. To study the role of myosin phosphorylation during muscle cell differentiation, we investigated precursor cells to skeletal muscle, namely proliferative myoblasts. The Yaffe

L-5 810 line of cloned proliferative rat myoblasts is an actively dividing cell line in culture which under the appropriate conditions can form myofibrils. We now report: (1) that myosin isolated from proliferative myoblasts contains light chains of 20,000 and 15,000 MW, (2) the 20,000 MW light chain can be phosphorylated by an endogenous myosin light chain kinase[14], (3) the proliferative myoblast myosin light chain kinase does not require calcium ions for activity, and (4) phosphorylation is a pre-requisite for actin activation of the myosin's ATPase activity.

Myosin and the myosin light chain kinase were isolated from 3 g of proliferative rat myoblasts by extraction at high ionic strength (0.5 M KCl, 15 mM Tris-HCl (*p*H 7.5), 10 mM $Na_4P_2O_7$, 10 mM dithiothreitol (DTT), 1 mM EDTA), followed by dialysis against low salt (0.05 M KCl, 10 mM Tris-HCl (*p*H 7.5), 5 mM DTT, 0.1 mM EDTA) to form a precipitate and sedimented at 48,000*g* for 10 min. The pellet was suspended in 3 ml of 0.5 M KCl, 15 mM Tris-HCl (*p*H 7.5), 1.0 mM EDTA, 2.5 mM DTT (protein concentration 5–10 mg ml^{-1}) and then made 10 mM in MgATP and fractionated with ammonium sulphate.

When the 35–55% saturation ammonium sulphate fraction was dialysed to remove the ammonium sulphate and then incubated with $MgAT^{32}P$, incorporation of ^{32}P was observed in the fraction as is shown in Fig. 1. Aliquots of the phosphorylated fractions at different times of incorporation were electrophoresed in 1% sodium dodecylsulphate (SDS)–7.5% polyacrylamide gels, stained for protein, and the entire gel was cut into slices which were then counted for ^{32}P. As Fig. 1 shows, within 30 min 0.8 mol of ^{32}P were incorporated per mol of P-light chain. Thus it was determined that an endogenous myosin light chain kinase was present in the proliferative myoblast actomyosin fraction.

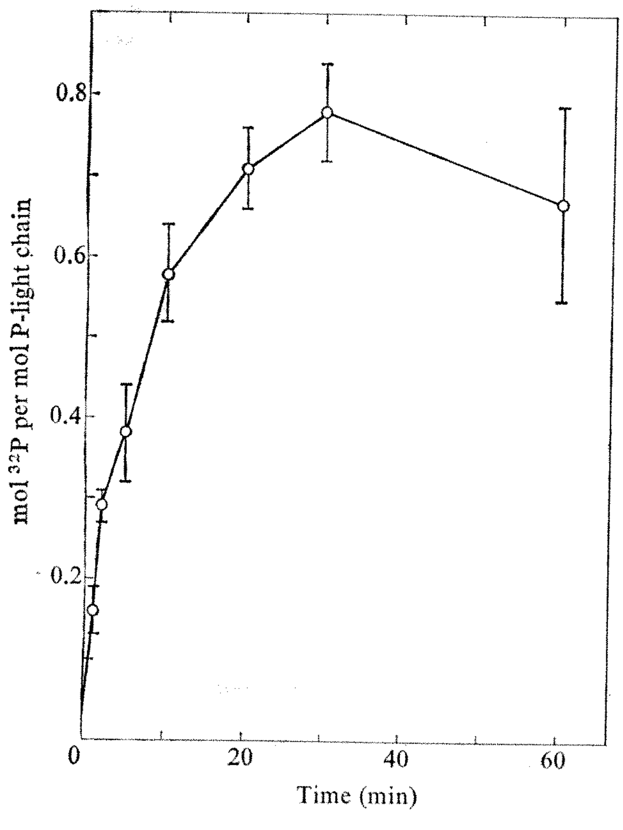

Fig. 1 Time course of ^{32}P incorporation into the proliferative myoblast myosin P-light chain by the endogenous myosin light chain kinase. The 35–55% saturation $(NH_4)_2SO_4$ fraction of actomyosin was dialysed against 0.5 M KCl, 15 mM Tris-HCl (*p*H 7.5), 2.5 mM dithiothreitol (DTT), 1 mM EDTA and incubated in the phosphorylation conditions: 0.2 M KCl, 30 mM Tris-HCl (*p*H 7.5), 12.5 mM $MgCl_2$, 5 mM DTT, 0.2 mM $CaCl_2$, and 0.1 mM ATP. The ATP was premixed with $Na_4\gamma$-$AT^{32}P$ (30 Ci $mmol^{-1}$) so as to give 200,000 d.p.m. in 5 µl of the final mixture (0.4 ml). The reaction was stopped by the addition of SDS to a final concentration of 1% and 0.05% $Na_4P_2O_7$. These samples were then dialysed to equilibrium against 0.3 M, 0.2 M, 0.1 M NaCl, and finally deionised water to remove the excess $AT^{32}P$. 1% SDS–7.5% polyacrylamide gel electrophoresis was carried out as described previously[16] with the exception that diallyltartardiamide (BioRad) was substituted for *bis*-acrylamide in the same molar ratio to the acrylamide[17]. The P-light chain was cut out of the gel and dissolved with 2% periodic acid and then counted in ACS (Amersham/Searle) scintillant. The data are for four determinations from three different preparations ± s.d.

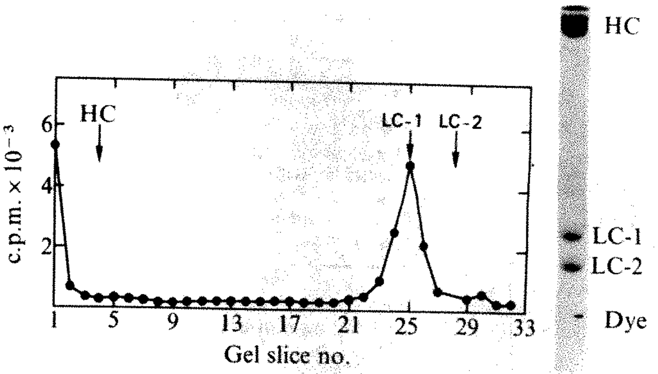

Fig. 2 Incorporation of ^{32}P into column-purified proliferative myoblast myosin with column-purified proliferative myoblast light chain kinase. Unphosphorylated myoblast actomyosin (35–55% $(NH_4)_2SO_4$ fraction) was made 10 mM in MgATP immediately before fractionation on a Sepharose 4B column (1.5 × 90 cm), which was equilibrated and eluted with 0.5 M KCl, 15 mM Tris-HCl (*p*H 7.5), 1 mM EDTA, 2.5 mM DTT. The 4 °C temperature, high ionic strength (0.5 M KCl), and speed of application of the sample to the column were necessary to inhibit phosphorylation in the unphosphorylated actomyosin. The myosin and myosin light chain kinase eluted as distinct peaks, were pooled individually, and then aliquots were mixed in the phosphorylation conditions and processed for gel electrophoresis as in Fig. 1, except that 5 µl of the final mixture gave 40,000 d.p.m. There were 3 µg of the P-light chain on the gel. The myosin light chain kinase fraction was purified only partially by gel filtration. But, only catalytic amounts of this fraction were used for the phosphorylations, as can be discerned by this gel, since no band at 80,000 MW characteristic of either the skeletal[7] or platelet[8] kinase can be detected. In similar experiments, the samples were treated with RNase, which eliminated the counts at the top of the gel, but did not change the incorporation into the 20,000 MW light chain. HC, myosin heavy chain (200,000 MW); LC-1, myosin light chain one, the P-light chain (20,000 MW); LC-2, myosin light chain two (15,000 MW).

Gel filtration of the 35–55% ammonium sulphate fraction on Sepharose 4B resulted in separation of purified myosin from the kinase (see refs 5, 3 for similar results with platelet and smooth muscle actomyosin). An SDS–polyacrylamide gel of such a pooled myosin fraction is shown in Fig. 2. The apparent MWs in SDS of the light chains were 20,000 (LC-1) and 15,000 (LC-2), similar to other proliferative myoblast myosin[15]. When this purified myosin fraction was incubated with the myosin light chain kinase fraction from the same column in the phosphorylation conditions (see Fig. 1 legend) and then electrophoresed on an SDS–polyacrylamide gel, incorporation of ^{32}P was seen only in the 20,000 MW light chain, the P-light chain. The amount of incorporation depicted in this figure is 0.76 mol of ^{32}P per mol of P-light chain. As controls, these two fractions were incubated separately in the phosphorylation conditions and subjected to gel electrophoresis as in Fig. 2. No incorporation was seen in either of these fractions.

A major difference between the kinase present in rat proliferative myoblasts and adult rat thigh muscle is shown in Table 1. Whereas the adult rat muscle kinase requires Ca^{2+} for its activity, the myoblast kinase does not. In this respect the myoblast myosin light chain kinase is of the cytoplasmic type, similar to those of human platelet[8] and mouse astrocytic neuroglial myosin[2]. The rat thigh muscle kinase is similar to that of rabbit skeletal muscle in requiring Ca^{2+} for activity[7].

The effect of phosphorylation on the specific ATPase

Table 1 Effect of calcium on the activity of the myosin light chain kinase

Kinase	Substrate	mol ^{32}P incorporated per mol P-light chain $+Ca^{2+}$	$-Ca^{2+}$
Proliferative myoblast	Myoblast myosin	0.75	0.81
	Skeletal myosin light chains	0.91	0.86
Rat thigh muscle	Skeletal myosin light chains	0.93	0.01

Either proliferative rat myoblast or adult rat thigh muscle myosin light chain kinase was column-purified as described in Fig. 2. These kinases were then incubated for 60 min under the phosphorylation conditions ($+Ca^{2+}$) as in Fig. 1 or in the presence of 2 mM EGTA ($-Ca^{2+}$) with native myoblast myosin or myosin light chains from rabbit skeletal muscle isolated from a 35–55% $(NH_4)_2SO_4$ fraction according to the method of Perrie and Perry[18]. All the values are calculated from 1% SDS–10% polyacrylamide gels as described in Fig. 1.

activities of phosphorylated and unphosphorylated proliferative myoblast myosin is shown in Table 2. To obtain phosphorylated and unphosphorylated myosin the 35–55% ammonium sulphate fraction was divided in half and was either phosphorylated or incubated without ATP and then chromatographed on a Sepharose 4B column. The purified myosin was pooled and the ionic strength was lowered to 20 mM KCl by dialysis. No actin activation of the myosin ATPase activity at low ionic strength (0.017 M KCl) can be demonstrated for the unphosphorylated myosin (Table 2). But a 9–12-fold increase in the myosin ATPase with actin is seen for the phosphorylated myosin. No effect of phosphorylation was seen on the high salt (0.5 M KCl) ATPase activity of myosin alone. Therefore, phosphorylation specifically affects the actin interaction with myosin.

Table 2 also demonstrates that even though the actin concentration is tenfold greater than the myosin concentration (mg per mg), there is no increase in the actin-activated ATPase activity on the addition of actin to unphosphorylated myosin. Using platelet myosin[4], a small amount of actin-activation of the myosin ATPase activity in the control (unphosphorylated) sample has been observed. In that work, it was unclear whether this actin activation was due to a small amount of phosphorylation or was a property of the non-phosphorylated myosin. In this report we show that before phosphorylation no actin activation of the myosin ATPase activity occurs (see Table 2) indicating that in the proliferative myoblasts, myosin phosphorylation is a prerequisite for actin activation. Further Table 2 shows that there is no difference in the actin-activated ATPase activity of phosphorylated myosin measured in the presence of Ca^{2+} and EGTA. This is similar to the results reported for phosphorylated platelet myosin[5], but differs from the findings for vas deferens myosin[3].

Table 2 The effect of phosphorylation on proliferative myoblast myosin ATPase activity

	Actin-activated ($+Ca^{2+}$)* $-$Actin	$+$Actin
Phosphorylated	0.015	0.175
Unphosphorylated	0.018	0.019
	Actin-activated ($-Ca^{2+}$) $-$Actin	$+$Actin
Phosphorylated	0.019	0.181
Unphosphorylated	0.020	0.022
	Myosin alone† K^+–EDTA	Ca^{2+}
Phosphorylated	1.31	0.218
Unphosphorylated	1.28	0.225

Specific activity is expressed as µmol Pi per mg myosin per min at 37 °C. The standard deviations of these values are less than or equal to 15%. Actomyosin fractions of proliferative rat myoblast myosin (see Fig. 1) were divided in half. One half was phosphorylated and the other half was incubated in the phosphorylation conditions without ATP. These fractions were then column-purified (see Fig. 2). These fractions gave similar electrophoretic patterns to those of Fig. 2. The data presented are from six determinations on four different preparations. Inorganic phosphate liberation was measured by a modification of the method of Martin and Doty[4,20]. In some experiments phosphate release was measured as ^{32}P released from AT^{32}P using the same method[20], but without the development of colour. Protein concentrations were estimated by the method of Lowry *et al.*[21] using bovine serum albumin as a standard.

*Assay conditions: 17 mM KCl, 10 mM Tris-HCl (*p*H 7.4), 1 mM ATP, 1.4 mM $MgCl_2$, 0.1 mM $CaCl_2$ ($+Ca^{2+}$) or 1 mM EGTA ($-Ca^{2+}$). Myoblast myosin 0.04–0.10 mg ml^{-1}, rabbit skeletal muscle actin[19], 0.4–1.0 mg ml^{-1}.

†Assay conditions: 0.5 M KCl, 20 mM Tris-HCl (*p*H 7.4), 2 mM ATP, and either 2 mM EDTA or 10 mM $CaCl_2$. Myoblast myosin, 0.04–0.07 mg ml^{-1}.

Myosin phosphorylation has been demonstrated previously to increase the actin-activated ATPase activity of platelet and smooth muscle myosin. The extension of this regulatory system to proliferative myoblasts, as well as the identification of myosin light chain kinase in astrocytic neuroglial cells[2] and HeLa cells (J. A. Trotter, S.P.S. and R.S.A., unpublished) suggests that myosin phosphorylation is a general regulatory mechanism controlling the actin–myosin interaction in both non-muscle and smooth muscle cells.

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A synthetic polypeptide with antifreeze activity

MUCH information has been gathered in recent years on the so-called 'antifreeze' proteins which lower the freezing point of the serum of certain marine fishes living in sub-zero water temperatures[1–4]. The proteins from the Antarctic fish *Trematomus borchgrevinki* are glycoproteins with a repeating alanyl-alanyl-threonyl tripeptide sequence, the threonyl residue being linked to a disaccharide[1,2]. In contrast, the antifreeze protein from the winter flounder *Pseudopleuronectus americanus* in the North American Atlantic coastal region is made up of eight amino acids with no apparent repeating sequence of the residues and no sugar moiety (ref. 4 and unpublished work of C. L. Hew, C. C. Yip & G. Fletcher). The antifreeze activity of these proteins is not compatible with the known colligative properties of solutes in solution and the mechanism of their action is not yet fully understood. But a common feature of both types of the antifreeze proteins is the preponderence of alanine which accounts for over 60% of the total amino residues. This fact, together with the absence of the carbohydrate in the protein from the winter flounder, prompted us to attempt the synthesis of polypeptide analogues having comparable proportions of alanine in them along with suitable other amino acids. As a first step, we made use of the lack of any obvious periodicity in the distribution of the alanyl residues in the flounder's protein and attempted the synthesis of a random copolypeptide containing about 65 mol % of alanine and 35 mol % of aspartic acid. The choice of aspartic acid was made on the basis of its being the next major amino acid in the flounder's protein[3,4] and on the expectation that its polar character will help the water-solubility of the alanine-rich copolypeptide, as in other studies on alanine-containing random copolymers. In addition, Duman and DeVries[4] have earlier indicated the involvement of carboxyl groups on the antifreeze activity by chemical modification studies. We report here the synthesis of this polypeptide and show that it possesses antifreeze activity.

The copolypeptide was synthesised by first polymerising the *N*-carboxyanhydrides of L-alanine and β-benzyl L-aspartic acid in the molar ratio of 65 : 35 respectively in dioxane with triethylamine as the initiator; the β-benzyl group of aspartic acid was then removed by using either anhydrous hydrogen bromide[6] or hydrogenfluoride–pyridine[7]. The products from both these procedures were very similar in their properties including the antifreeze activity.

Table 1 Properties of the synthetic polypeptide

Sample no.	Composition (mol 1 %)* Alanine	Aspartic acid	Molecular weight†	α-helical content‡
SAF-I§	65.5	34.5	45,000–70,000	15
SAF-II¶	64.8	35.2	50,000–70,000	20

*Determined on a Beckman Model 121M amino acid analyser after 24–48 h of hydrolysis in 12 M HCl at 110 °C. The compositions of the polypeptides before deblocking the side-chain benzyl group, were almost identical to those of the final products.

†Estimated using a Sephadex G-75 column calibrated with protein markers.

‡Estimated on the basis of the circular dichroism data as before[8].

§Synthesised using HF–pyridine debenzylation method.

¶Synthesised using the HBr debenzylation method.

Table 1 summarises the data on the amino acid composition, molecular weight distribution and α-helical content of the synthetic polypeptide. Compared with the flounder's protein, the synthetic analogue is found to have a larger average molecular weight and a much lower α-helical content: the molecular weight of the protein is about 10,000 and its α-helical content about 85% (ref. 8).

Figure 1 shows the antifreeze activity of the synthetic polypeptide plotted as a function of its concentration in 0.01 M KCl, *p*H 6.0: the corresponding data for the flounder antifreeze protein are also shown. The synthetic polypeptide is apparently 'active', that is, like the antifreeze proteins, it depresses the freezing point of the solution to a far greater extent than can be accounted for in terms of its colligative property. This is illustrated by the data shown in Fig. 1 for serum albumin (molecular weight 68,000), which does not exhibit any antifreeze activity. Other globular proteins like myoglobin and cytochrome *c* were also inactive. An interesting feature of the data in Fig. 1 is the indication of a plateau in the activity of the synthetic polypeptide at higher concentrations, again very similar to what is observed with the protein counterparts (refs 1 to 4 and unpublished work of C. L. H., C. C. Yip & G. Fletcher). The magnitude of the antifreeze activity of the synthetic analogue is about one-third that of the flounder's protein at a given concentration. The decreased activity of the synthetic polypeptide when compared with the protein may be due to the differences in their amino acid compositions, sequences and molecular weights as well as the secondary structure. But the fact that such a simple model polypeptide can, albeit partially, mimic the natural protein in its function, seems to indicate the importance of the predominance of alanine in all the known antifreeze proteins. While we do not have an immediate answer as to the role of either the alanyl or aspartic residue in the mechanism of action of these proteins, a systematic study of the properties of the synthetic polypeptide analogues will, as pointed out by Feeney[1], prove to be valuable in our understanding of these proteins. Such a study is now in progress in our laboratories.

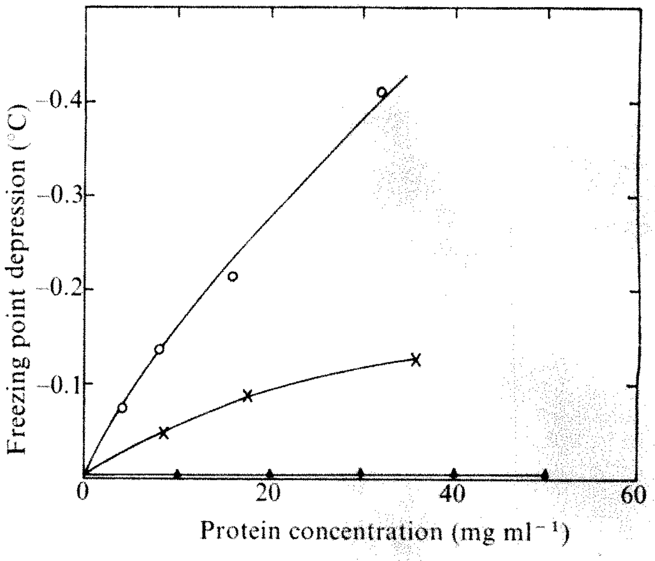

Fig. 1 Antifreeze activity of the synthetic random copolypeptide (×), SAF-I as a function of its concentration. The sample was dissolved in about 0.5–1.0 ml of 0.01 M KCl, *p*H 6.0, to yield the highest required concentration and dialysed extensively against the same solvent. The activity was determined as depression of the freezing point of the solution, using an Advanced Instruments model 3-R osmometer. Correction was made for the KCl in the solvent. The concentration of the polypeptide was determined by using $E^{1\%} = 18$ at 230 nm (ref. 8). The antifreeze activities of the flounder's protein (○) (unpublished work of C. L. H., C. C. Yip & G. Fletcher) and albumin (▲) are shown for comparison.

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Effect of light on artificial lipid membranes modified by photoreceptor membrane fragments

THE molecular basis of photoreception has been extensively studied, but it is not yet clear which of the photolysis products of rhodopsin has the key role in receptor potential generation. Penn and Hagins[1] have shown that the effect of an absorbed photon persists for about 0.1 s at 33 °C (rat retina). If metarhodopsin II is the key product, as most workers assume it to be, then the result of Penn and Hagins can be explained as follows. On formation of metarhodopsin II a visual pigment molecule undergoes a transformation with time constant 0.1 s, which 'cuts-off' the process of visual excitation. Metarhodopsin II persists for dozens of seconds at 33 °C (as determined from spectral characteristics[2]) so if the suggested transformation takes place it should not result in variations of the absorption spectrum of metarhodopsin II, and thus could not be detected by spectroscopy. As the artificial lipid membrane (ALM) is a good detector of changes in functionating protein molecules[3–5], we attempted to detect the above process by examining the photoeffect on ALM, modified by photoreceptor membrane components.

Such an approach seems to be verified also by the fact that rhodopsin is a typical membrane protein, and investigation of the above model might give information useful for understanding the mechanisms of receptor potential generation. We used ultrasonicated rod outer segment fragments (ROSF) for the modification.

ROSFs were obtained by ultrasonic treatment of ROS suspension in distilled water. The membranes were formed using the method of Mueller *et al.*[6] on a hole (1-mm diameter) of a Teflon cup placed in a glass chamber. The lipid solution used contained egg lecithin (18 mg ml^{-1}) and cholesterol (12 mg ml^{-1}) in heptane. The modification was carried out in the solution containing ROSF (absorbance 0.15 at 500 nm). The solutions on each side of the membrane were the same in all cases. A current through ALM was measured using an electrometer amplifier (Keithley model 301) in voltage-clamp conditions. The capacity of the modified ALM was two to three times lower than that of unmodified membranes (membrane capacity was evaluated from the time constant of the current recording circuit). The dark d.c. resistance of both modified and unmodified membranes was of the order of $5\times10^{10}\,\Omega$. 1-ms flashes from a xenon discharge tube were used to illuminate ALM.

The response of the modified membrane to a light flash is shown in Fig. 1 which shows that a flash brings about an increase of current through the membrane. A characteristic feature of the response is its reversibility—an increase of the current is followed by a spontaneous drop to the dark level. In the conditions of illumination used (bleaching of no less than 80% or rhodopsin), the effect could be observed only once for each modified membrane: repeatedly flashed white-light illumination caused no photoelectric current. Illumination by red light (beyond 650 nm) affected neither the current through the membrane, nor its ability to respond to a subsequent illumination by white light. In unmodified membranes, no photoeffect could be observed.

Further investigation of the photoeffect has shown the following:

(1) The effect was independent of purity of the preparations. In the case of low-purity ROS (the purity criterion measured according to Heller[7] being about 4.0), the membrane response was the same as with pure preparations (purity about 2.2). This indicates that a substance responsible for photosensitivity of ALM is contained in the outer segment.

(2) The direction of current through the membrane was determined by the applied voltage, and its magnitude always increased in response to light. At zero applied voltage, there was no current and no response. These facts show that the illumination of ALM brings about an increase in the membrane conductivity rather than the appearance of a photo-electromotive force, which had been observed for membranes containing carotenoids[8,9].

(3) Hydroxylamine at 100 mM did not change the observed photoeffect.

(4) A steady-state conductivity of a modified membrane was generally somewhat higher after illumination than before illumination.

(5) The photoeffect was observed, with no significant changes, in 100-mM NaCl and in 100-mM KCl (both solutions buffered with 10-mM imidazole/HCl, *p*H 6.25). The addition of Ca^{2+} to these solutions at 10 mM did not produce any detectable changes in the photo-induced conductivity. Addition of Ca^{2+} at higher concentrations resulted in a rapid aggregation of ROSF.

Investigation of the photoeffect in ALM at various temperatures gave the following results:

(1) The time constant of the photo-induced increase in the ALM conductivity was, within experimental error, the same as the period of formation of metarhodopsin II, cited in the literature (refs 10 and 11). An activation energy of the process was about 30 kcalorie mol^{-1}.

(2) The rate of the subsequent decrease in the model membrane conductivity depended on temperature according to the Arrhenius' law, with activation energy 19 ± 3 kcalorie mol^{-1} (Fig. 2). From the extrapolation of the Arrhenius' plot to 33 °C, the time constant of the process was evaluated to be 80 ± 10 ms. Our data thus indicate that in the modified ALM light induces a process with time constant close to the duration of "a photon effect" as determined by Penn and Hagins[1]. The observed return of the model membrane conductivity to a near dark level may therefore be con-

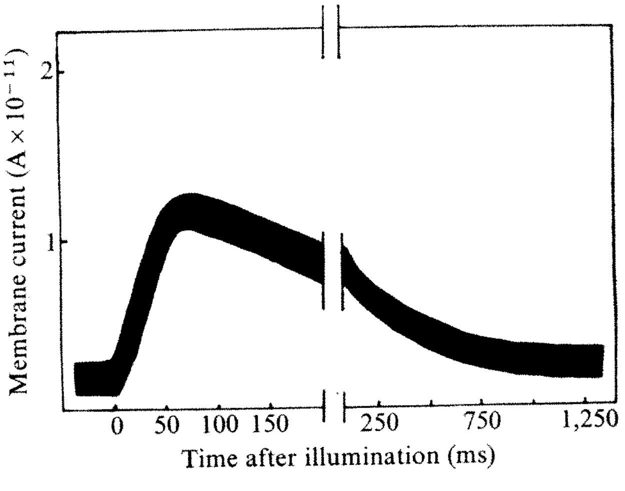

Fig. 1 The effect of illumination on artificial lipid membrane modified by ROSF. Conditions: 100 mM NaCl, 10 mM imidazole/HCl, *p*H 6.25; 17 °C. Voltage across the membrane, 100 mV. Recording time constant for the current flowing through the membrane—about 2 ms. ROS were isolated as follows: retinae, excised from dark-adapted cattle eyes, were put in isoosmotic buffer solution (140 mM NaCl, 3.5 mM KCl, 1.8 mM CaCl$_2$, 0.5 mM MgCl$_2$, 10 mM Tris, *p*H 7.4) identical to that used by Korenbrot and Cone[13], 1 ml per retina, and subjected to mild homogenisation on a magnetic stirrer for 20 min. The homogenate was centrifuged for 10 min at 100*g*. The supernatant was collected and centrifuged for 10 min at 10000*g*. The sediment was suspended in 40% (w/v) sucrose solution prepared in the isoosmotic buffer and subjected to flotation for 30 min at 30,000*g*. The flotation was repeated twice. To determine the optical density and the purity criterion, the ROS were solubilised with 2% solution of cetyl trimethylammonium bromide.

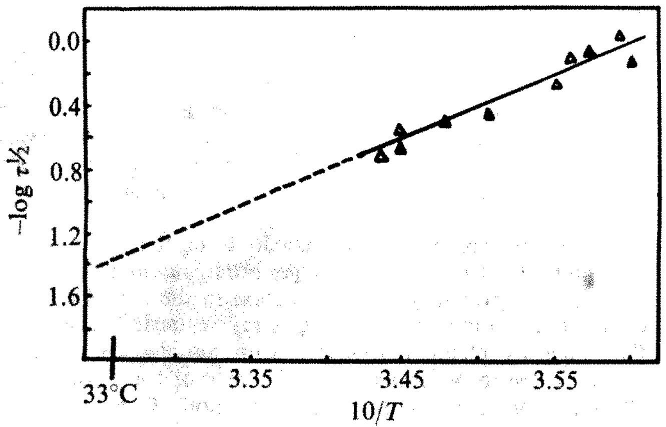

Fig. 2 Temperature (T) dependence of period (in seconds) required for the return of model membrane conductivity to half-maximum level ($\tau_{1/2}$).

nected with a 'switching off' of a mechanism inducing the receptor potential of a rod. No definite conclusions could be made as to the nature of the process involved, however. The mechanism probably involves either a conformation transition in rhodopsin after the formation of metarhodopsin II, or a rearrangement of photoreceptor membrane occurring, following a certain delay, after the formation of metarhodopsin II.

What is the mechanism of the observed photoeffect? The answer would depend on which situation was realised in our experiments—whether visual pigment molecules (or disk membrane fragments) are included in ALM, or ROSF in the shell form are simply sorbed at the model membrane. If the former is true, then our data apparently indicate that rhodopsin can modulate photoreceptor membrane permeability. In that case, one could suggest the simple realisation of a transmitter scheme of the photoreceptor function (for example, see ref. 12): a short-period photoinduced increase in the disk membrane permeability results in the release of a quantity of mediator capable of changing the permeability of ROS plasma membrane. If the latter version is the case, the variations in ALM conductivity most probably do not represent changes in disk membrane conductivity, and the model membrane would act only as a detector of rearrangements in visual pigment and (or) its lipid surroundings.

As yet it is impossible to make a choice between the two alternative mechanisms of photoeffect observed.

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Effect of aminoacylation on the conformation of yeast phenylalanine tRNA

MUCH effort has been devoted to analysing the effect of aminoacylation on the structure of transfer ribonucleic acid (tRNA). While it is generally held that changes in conformation provide the basis for the discrimination between acylated and nonacylated tRNA in tRNA: aminoacyl tRNA synthetase, tRNA: ribosome and tRNA: co-repressor interactions, the evidence to support this view to date is in serious conflict. We have been using the technique of laser light scattering to investigate the tertiary structure of tRNA (ref. 1). This technique is particularly well suited for analysing structural changes in macromolecules such as tRNA as it can detect changes of as little as 1% in the translational diffusion constant. The method has the advantage of allowing rapid, continual data monitoring making it possible to study dynamic changes in conformation. We describe here the use of this technique to monitor the diffusion coefficient of aminoacylated tRNA in conditions where deacylation occurs spontaneously. The results clearly demonstrate that: (1) large changes in the conformation of yeast phenylalanine tRNA do occur on aminoacylation, (2) the change can be reduced to an undetectable value by small changes in the solution parameters, and (3), the results apparently provide an explanation for many of the discrepancies observed by others.

Figure 1 shows the results obtained when phenylalanyl-tRNA was allowed to deacylate in the two different solution conditions. When the tRNA was in 10 mM Mg^{2+}, $D_{20,w}$ increased linearly from 7.46×10^{-7} cm^2 s^{-1} at 555 pmol per A_{260} to 7.79×10^{-7} cm^2 s^{-1} at 35 pmol per A_{260}. In this condition, the half-time for deacylation was 71 min. A least squares fit to these data yields an overall change in $D_{20,w}$ of $4.6 \pm 0.6\%$ with an extrapolated change of $14 \pm 2\%$ starting with pure, fully aminoacylated tRNA. On the other hand, when deacylation was allowed to occur in 1 mM $MgCl_2$ the values of $D_{20,w}$ showed no change as the extent of acylation decreased from 620 pmol per A_{260} to less than 1 pmol per A_{260}. In this condition the half-time of deacylation was 55 min. The slope of the plotted line was calculated to be zero within limits of error ($-0.5 \pm 0.9\%$).

The results show that the diffusion constant for yeast tRNA$^{\rm Phe}$ increases on deacylation in 10 mM Mg^{2+}. This change indicates that deacylation results in the formation of a more compact structure than that which obtains for the aminoacylated conformer. This interpretation agrees with results of Kaji and Tanaka[2] which indicated that aminoacylated *Escherichia coli* tRNA$^{\rm Phe}$ sediments slower than the uncharged species.

Of the interpretations consistent with our observations, the most plausible are that aminoacylation results in: (1) a disruption of secondary base pairing with resultant changes in secondary and tertiary structure or (2) changes in tertiary structure alone owing to a disruption of tertiary base pairing and/or an alteration in the distribution of counterions. The possibility that the observed changes in $D_{20,w}$ for tRNA$^{\rm Phe}$ in 10 mM Mg^{2+} is simply due to the addition of a molecule of phenylalanine can be ruled out due to the large magnitude of the change. The first interpretation also seems unlikely in light of results from a number of circular dichroism studies performed under a variety of solution conditions which indicate that no changes occur in the secondary structure on aminoacylation[3–6].

Alteration of the tertiary structure alone thus seems most plausible and while the results of this experiment do not allow us to specify the portion(s) of the tertiary structure involved in the change, results from other studies provide some insight. Two studies have shown increased steroid[7] and polycytidine[8] binding to the aminoacylated form of many tRNAs. Both ligands selectively bind guanosine residues suggesting that aminoacylation exposes the pair of guanosine residues which occur in the dihydrouridine loops of nearly all tRNAs[9]. In addition, results from studies on the binding of complementary tetranucleotides to yeast $tRNA^{Phe}$ strongly suggest that residues in the dihydrouridine and pseudouridine loops are exposed on aminoacylation[10].

Other results indicate that changes can also occur in the region of the junction of the D-stem and amino acid acceptor stem. Differences were found in the circular dichroism band associated with 4-thiouracil when the spectra of charged and uncharged *E. coli* $tRNA_F^{Met}$ were compared[11]. EPR studies with *E. coli* $tRNA^{Phe}$ have shown a difference on aminoacylation of the rotational correlation times from a spin label attached to 4-thiouracil[12].

Taken together, these results suggest that aminoacylation may result in the disruption of tertiary base pairs between the dihydrouridine and pseudouridine loops and rearrangement of the 'L' structure to a less compact conformation. An apparent exception to this interpretation comes from results of a nuclear magnetic resonance study which showed no changes in hydrogen bonding of yeast $tRNA^{Phe}$ on aminoacylation[13]. This study was performed at *p*H 5.0, however, a condition in which others could not detect a change in sedimentation behaviour on aminoacylation[14].

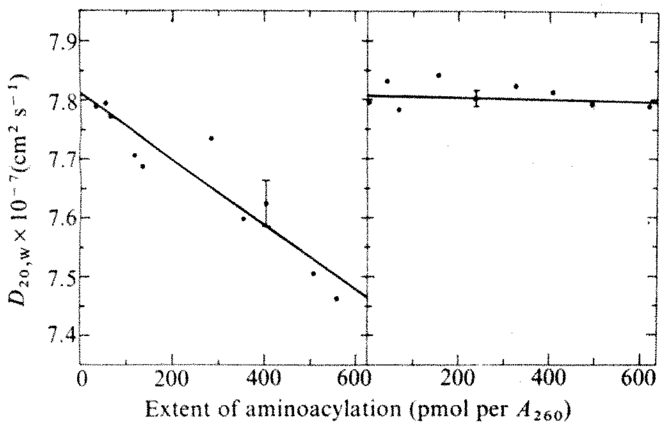

Fig. 1 Translation diffusion constant ($D_{20,w}$) of yeast $tRNA^{Phe}$ as a function of the extent of aminoacylation. Phenylalanine tRNA with an acceptor activity of 850 pmol per absorbance unit (A_{260}) was aminoacylated with ^{3}H-phenylalanine (200 mC mmol^{-1}) in a reaction mixture that contained, per ml: 25 µmol Tris-HCl, *p*H 7.2, 12.5 µmol $MgCl_2$, 4 µmol ATP, 5 nmol ^{3}H-phenylalanine, 2 nmol $tRNA^{Phe}$ and sufficient phenylalanyl-tRNA synthetase to allow complete charging in 15 min at 37 °C. When acylation was complete, 0.1 volume of 20% potassium acetate, *p*H 4.5, was added to the reaction mixture and the aminoacyl-tRNA repurified by chromatography on a small column of DEAE–cellulose. The tRNA was precipitated with ethanol, recovered by centrifugation, dried under vacuum and stored at −20 °C until use, usually within 18 h. In a typical experiment, 5 A_{260} (250 µg) of charged $tRNA^{Phe}$ were solubilised in 25 µl of 1 mM sodium acetate, *p*H 4.5 and dialysed against the same buffer with 1.0 or 10 mM Mg^{2+}. One-fifth volume of a 5 × stock of the appropriate buffer was then added: 8 µl of the sample were filtered into a 1 × 1 × 45-mm cuvette for light scattering; the remainder was filtered into a test tube thermostatically maintained at the same temperature (20 °C). Portions of the larger sample were withdrawn at various times, acidified with trichloroacetic acid and the acid-insoluble radioactivity collected on glass fibre filters to determine the extent of deacylation. Details of the procedures used in the purification of the tRNA and synthetase, the preparation of the ^{3}H-phenylanyl-tRNA and the preparation of the tRNA for light scattering will be described elsewhere. The techniques for data collection and analysis have been described[1]. The concentration of tRNA for the experiment done with 10 mM Mg^{2+} was approximately twice that used for the measurement taken at 1 mM Mg^{2+}. Hence, extrapolation of the diffusion constant values to zero tRNA concentration would yield a higher value with 1 mM Mg^{2+} than with 10 mM Mg^{2+}. That extrapolation was not done here because an unknown, but constant amount of stray light was present due to the very small size of the cuvettes used for the measurements. The effect of stray light is to lower observed diffusion constant values by about 2% for every 1% of stray light present and is more significant at low concentrations of tRNA where the relative contribution by the stray light increases. These effects were not important in the large cuvettes used in previous experiments[1]. Measurements were made at 20 °C for samples in 0.01 M Tris-HCl (*p*H 7.2), 0.1 M NaCl with 10 mM $MgCl_2$ and 6.2 mg ml^{-1} tRNA (*a*) or 1 mM $MgCl_2$ and 3.0 mg ml^{-1} tRNA (*b*). Each point represents an average of at least six determinations made within a 10-min period. Values of diffusion constant are normalised to 20 °C and the viscosity of pure water. Error bars represent ±1 s.d. (σ) about the point; σ showed little variation during the experiment, with values consistently less than 0.3% of $D_{20,w}$.

Less likely, because of the magnitude of the effect, is the possibility that the observed changes occurring on aminoacylation are due primarily to an alteration in the distribution of counterions. Ninio *et al.*[15] using X-ray scattering, concluded that intensity changes attendant with aminoacylation were probably due to perturbations in the layer of counterions—an effect eliminated at low Mg^{2+} concentrations. Consistent with this view, Cohn *et al.*[16] found that the number of manganese binding sites changed with aminoacylation. Thus, it is possible that aminoacylation results in minor rearrangements of structure which alter divalent cation binding sites. This in turn could affect the distribution of counterions and hence, the diffusion constant. These interpretations are also consistent with the negative results obtained in a number of studies designed to detect changes in secondary structure[3–6,13,17–19].

The results presented here do not allow us to discriminate between changes in tertiary structure and/or counterion distribution. It is, however, possible to determine the contribution of each effect on the diffusion constant by performing this experiment at various concentrations of tRNA and salt. These experiments are in progress. The present results vividly demonstrate the importance of Mg^{2+} ions in structural changes of $tRNA^{Phe}$ on aminoacylation. Magnesium ions have been implicated previously in structural changes of deacylated $tRNA^{Phe}$ (ref. 1); however, this is the first clear evidence that the conformational changes which occur on aminoacylation/deacylation are altered by magnesium. The results also suggest that specific changes in intracellular magnesium levels might have a central role in regulation of tRNA function.

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matters arising

The Star of Bethlehem

I WOULD like to make a brief comment on David Hughes's article on the Star of Bethlehem. While the astronomical phenomena are described with vivid precision, the historical aspect would merit equal attention. Dionysius Exiguus is surely thought to have placed the Annunciation at 25 March 1 BC and the Nativity therefore at 25 December 1 BC and not AD 1. I quote from E. J. Bickerman[1] in support of my view that Dionysius' supposed 5-yr error is at best an irrelevance (page 77 of ref. 1).

> An era *ab urbe condita* (from the founding of the city of Rome) did not in reality exist in the ancient world, and the use of reckoning the years in this way is modern. . . . The principal reason for not using the system *ab urbe condita* was that the age of the city was disputed: *est enim inter scriptores de numero annorum controversia* (Cic. *Brut.* 18, 72). The date of the founding in Roman historiography—excluding the more extreme opinions—oscillates between 759 and 748 BC.

Of 58 modern authors cited by Holzmeister[2], 33 select for the year of the Nativity 7–5 BC, so Hughes represents majority modern opinion. Of 42 ancient opinions listed, however, not one falls as early as this period and only one before the supposed date of Herod's death. A thorough re-examination of the date of the Nativity must also ask whether it is certain that Herod died in 4 BC. Josephus states that Herod reigned 34 years from the death of his predecessor, executed by Antony some time after the capture of Jerusalem which fell in 37 BC. The obvious arithmetical error is accounted for by the explanation that Josephus counted the year in which a king died and the (same) year of the accession of his successor as two separate years, and therefore, overestimated the length of each reign by 1 year—his so-called 'inclusive' method.

Although this notion of Josephus' mode of computation is the pillar on which the modern opinion rests, no shred of evidence is adduced for it, for there is none. Evidence to the contrary is, however, easily found in *Jewish Antiquities*. Between February 135 BC and October 63 BC there were in Jerusalem six rulers in the 72 years 8 months. Summing the lengths of reigns as given by Josephus, we have 71 years 6 months. This is very exact and certainly evidence that Josephus did not reckon 'inclusively'.

Since Herod died shortly after a lunar eclipse, the times are restricted in which his death could have fallen. 4 BC is too early to allow for a reign of 34 years (if the 'inclusive' method of counting is rejected) and there were no such eclipses in 3 or 2 BC. It is then hard to escape from the conclusion that Herod died following a partial eclipse in 9/10 January 1 BC having completed 34 years' reign a few months earlier. Just before the eclipse, there was an uproar in the Temple recorded by Josephus, and possibly unleashed by the advent of the Magi if the traditional date of the Adoration, 6 January is again considered.

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[1] Bickerman, E. J. *Chronology of the Ancient World* (Thames and Hudson, London, 1968).

[2] Holzmeister, U. *Chronologia Vitae Christi* (Rome, 1933).

HUGHES'S suggestion that the 'Star of Bethlehem' was probably a triple conjunction of Saturn and Jupiter in the constellation Pisces in 7 BC was anticipated by Stauffer in 1957 (*Jesus: Gestalt und Geschichte*). The significance of this *'coniunctio magna'* was explained as follows: Jupiter was the 'star' of the world ruler and the constellation of the fishes as the sign of the last days; the planet Saturn was considered in the east to be the 'star' of Palestine. So the conjunction indicated, not a 'Jewish king born in Israel', but that in that year there would appear in Palestine the ruler of the world in the last days.

As long ago as 1835 D. F. Strauss observed the evangelist's contrived story to show fulfilment of the prophecy of Micah that the ruler of Israel should come from Bethlehem, and claimed "we have absolutely no guarantee that Bethlehem was his birth-place" (*The Life of Jesus Critically Examined*, SCM ed. 1973, p 156). Guignebert concluded that the magi, star, massacre of innocents, birth in a stable, shepherds, angels, and so on, are all unhistorical and that we do not know where Jesus was born, except in Galilee. He also noted that the idea that the Jewish population had to move all over the land to register for the taxation was "outside the plane of reality" (*Jesus* 1935, pp. 94 & 99). Mackinnon noted the 'assumption' that enrolment required the parents of Jesus to journey to Bethlehem for this purpose, and did not believe the stories of the magi and the massacre of infants (*The Historic Jesus*, 1931, pp. 19 & 24). More recently, Gunther Bornkamn, in his *Jesus of Nazareth* (1951) says hardly anything about the birth, infancy or early years of Jesus. He claims that the birth and infancy stories of Matthew and Luke are largely legendary in character and do not contribute to a *History* of Jesus.

All that we know about Jesus is that he was born in Galilee. If he was about 30 years old (Luke III, 23) when John baptised him in Tiberius's 15th year (Luke III, 1) then it looks as if he was born in the terminal year, and that the chronology of Dionysius Exiguus is correct. We know of no reason why this should not have been so, and there is no cause to believe the stories associating the birth with celestial signs or the Roman registration of 6 CE.

Incidentally, Dionysius did not "forget the year zero"; there was no such year, as demonstrated by Hughes's own chart!

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KEPLER may not have been the first to be impressed by a Jupiter–Saturn appulse[1]. In the chronicles of Worcester Priory (1377) a conjunction in Aquarius was recorded in 1285—"it had not happened since the Incarnation". Newton[2] comments that this suggests that the Star "had already been interpreted as a conjunction by AD 1285". It could be a rhetorical expression.

The object of −5 (6 BC) is regarded by Stephenson[3] as a comet, because there is a possible reference to a tail and motion and certainly there does not seem to be any remnant known.

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[1] Hughes, D. W. *Nature* **264**, 513–517 (1976).

[2] Newton, R. R. *Mediaeval Chronicles and the Rotation of the Earth* 691 (John Hopkins University Press, Baltimore and London, 1972).

[3] Stephenson, F. R. *Q. Jl R. astr. Soc.* **17**, 12 (1976).

I COMMENT here on two points made by David Hughes. First, there seems to be no need for a year zero because from our standpoint, in 1976, any year in which Jesus Christ was born, be it spring or autumn, was 1 AD—"the first year of the Lord," and the preceding year 1 BC—"the first year before Christ." Perhaps the author thinks of

1 AD as the first year after the birth of Christ.

Second, for those of us who hate to believe that St Luke or any Holy Scripture could have been "mistaken", or at least so easily written off as such, perhaps Cyrenius and Quirinius are not one and the same.

With no year zero to account for between 1 BC and AD 1, the author's second scenario seems more likely to conform to fact.

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HUGHES, in his article on the Star of Bethlehem, makes life unnecessarily difficult for himself. He estimates that a journey of 550 miles across the desert, from Sepharuaim to Jerusalem would have taken four months in preparation and travelling. Consequently he has to show that the triple conjunction of Jupiter and Saturn in Pisces would have been visible, in varying positions, throughout that period of time.

T. E. Lawrence, writing of the Arab Revolt in *Seven Pillars of Wisdom*, recorded that a fully loaded camel, under an experienced rider, could, if hard pressed, cover 80 to 100 miles in a 24-hour period, depending on the country, and that 50 miles ridden in the same period was considered a holiday by comparison. Even the most inexperienced and clumsy riders, who positively hindered their animals, could travel 30 miles a day. The length of stages undertaken in the crossing of a desert is limited, of course, by the distance between wells, but even so, the Magi could have completed their journey from Babylonia in 2 weeks at the outside, or in 10 days with less comfort. Allowing 2 weeks for preparation (gathering of stores, hiring of men and beasts and so on), the whole enterprise would have taken a month at the most.

In other words, the Magi not only could have set off when the triple conjunction was at its best, but would also have been in a position to respond to signs of a much more transient nature.

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HUGHES[1] convincingly dates Jesus' birth by astronomy to 7 BC but unfortunately rejects the historical evidence in Luke II, 2, as usually found in English versions, when an equally valid and grammatically justified alternative translation can be used to support his theory. Luke II, 2 is usually translated [illegible] the first enrolment when Quirinius was governor of Syria"[2]. An enrolment, or census, under Quirinius is well documented because of a subsequent rebellion[3–5], but Quirinius was not governor until AD 6–9. Thus, Luke seems to contradict both the astronomy and Matthew (II, 1), who says Jesus was born before Herod the Great died, that is, before 4 BC.

The necessary change, which completely alters Luke's evidence depends on the use of the Greek word *protos*, usually translated 'first'. *Protos* is the superlative derived from *pro*, meaning before, the comparative being *proteros*, meaning former or sooner. Consequently, according to strict grammar, *protos* should mean first of at least three. But the Hellenistic Greek used in New Testament times was at least as relaxed as modern English in which 'first' is often used when 'former' or 'prior' would be more grammatical[6]. Thus, 'first enrolment' in its Hellenistic connotation is 'first of two' or 'one before' and, as Bruce[7] suggests, Luke II, 2 is best translated "This enrolment was before that made when Quirinius was governor of Syria". Similar expansions, in which words need to be supplied in translation, are not uncommon and there is no grammatical reason against supplying them in this passage[6].

Numerous Egyptian papyri of a slightly later date indicate that enrolments occurred every 14 years (ref. 3). If this 14-year interval was observed in Syria the enrolment before that of Quirinius would have been sometime between 9 and 6 BC. Thus, Luke II, 2, instead of being dismissed as a mistake, can be used to support the date deduced by Hughes.

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[1] Hughes, D. W. *Nature* 264, 513–517 (1976).
[2] *Bible, Revised Standard Version* (quoted as a modern literal translation).
[3] Souter, A. & Baker, J. C. in J. Hastings *Dictionary of the Bible* 2nd edn (Clark, Edinburgh, 1963).
[4] Bruce, F. F. *New Testament History* 90–91 (Nelson, London, 1969).
[5] Acts, V, 37, also written by Luke.
[6] Turner, N. *Grammatical Insights into the New Testament* 23–24 (Clark, Edinburgh, 1963).
[7] Bruce, F. F. *New Testament History* 30 (Nelson, London, 1969).

HUGHES REPLIES—The significance of the triple conjunction of Jupiter and Saturn in the constellation of Pisces[1] around 7 BC can be beter judged by reference to Fig. 1. At no time do the planets approach closer than 1 °, about twice the diameter of the full moon. All the stars in this region pale into significance in comparison with the brightness of the two planets (see Fig. 2).

'Herod' was a fairly common name in those times. Herod the Great, the son of Antipater, was made the Governor of Galilee anud afterwards titular King of Palestine by the Romans. On his death the kingdom was divided between his three sons, Archelaus, Herod Antipas (who received Galilee) and Herod Philip II. There is very little doubt that Jesus was born during the reign of Herod the Great (Matthew II, 22) and was tried and crucified during the reign of Herod Antipas (Luke XXIII, 8, Matthew XIV, 1); however, some biblical chronologists do cast doubt on the generally accepted superstition that Herod the Great died between 13 March and 11 April 4 BC, as Edwards shows above.

Simmons finds the month of the birth of Christ by relating the lives of Christ and John the Baptist (personal communication). Zacharias, the father of John the Baptist, was a priest of the course of Abia (Luke I, 8) and would have served in the temple during the 6th week after Passover, the week before Pentecost. As all the priests also served during Pentecost, Zacharias would have left Jerusalem for his home town around Sivan 12th (15 June). Elizabeth, his wife, conceived soon after his return (Luke I, 24) so John the Baptist would have been born about 280 days later, around 27 March. Luke (I, 36) records that Christ was 6 months younger than John the Baptist so this puts the birth of Christ in late September. Interestingly we read in the Talmud that sheep were taken in from the hills on about the first of November until March so shepherds would not be on the hills watching over their sheep between these dates.

Addey[5], using the same evidence as Hughes[1] comes to a similar conclusion, that Christ was born in the Autumn of 7 BC. Using traditional astrology (apparently giving Christ's horoscope a strong Sun/Leo/fifth house element) and the early Christian tradition that Jesus was born on the day after the Jewish Sabbath, that is on a Sunday, Addey finds the characteristics of Jesus are best fitted by the horoscope for the evening of Saturday 22 August 7 BC (note that in the Jewish calendar the

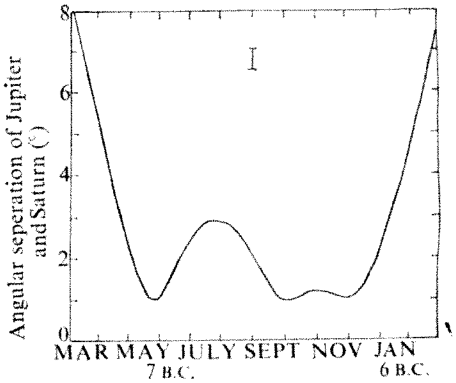

Fig. 1 The angular distance between Saturn and Jupiter during the triple conjunction of 7 BC. The scale bar represents the diameter of the moon in the same scale.

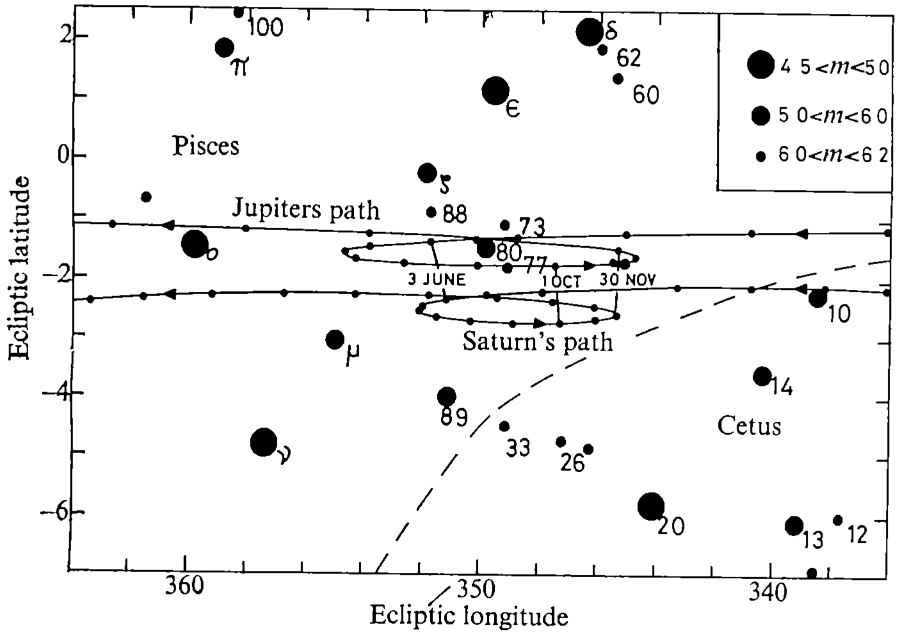

Fig. 2 The 7 BC triple conjunction of Saturn and Jupiter in Pisces. The ecliptic latitude and longitude have been taken from Tuckerman[2]. The dots on the planetary paths are separated by 20-day intervals; *m* is the apparent stellar magnitude.

day changes at sunset). The legend that Christ was born shortly after sunset, which originates from the Arabic Gospel of the Infancy, is also mentioned by Kestranek[6]. The mystical writings in The Urantia Book[7] give the time of Christ's birth as noon on 21 August 7 BC.

A novel hypothesis that has also been considered is that there were two Messiahs, both named Jesus born at slightly different times (ref. 8). Edwards supports this, pointing out that the nativity in Luke belongs as firmly to the reign of Archelaus as the Mathean nativity does to the reign of Herod the Great (O. Edwards, personal communication). Steiner[8] maintains that Matthew describes the birth of one Jesus, who was visited by the Maji whilst Luke describes the birth of a second Jesus who was born a little later and was visited by the shepherds and eventually crucified. The evidence he brings forward to support this claim is that Matthew and Luke give different genealogies, in Matthew Jesus being descendant from Solomon whilst in Luke He is traced back to Nathan. Second, in Matthew Jesus is born in a house and is visited by wise men, in Luke He is born in a stable and visited by shepherds. Third, Matthew describes the slaughter of the innocents and the flight into Egypt whilst this is not mentioned at all in Luke. This plurality of Messiahs was apparently concealed by Jerome and the newly official Church of the Roman Empire when they juggled with the different genealogies and expurgated the Infancy Gospels to produce a simple creed for the masses on whom the Church of the Roman Empire had been newly imposed. The expectation of the coming of two Messiah was often mentioned in the Dead Sea Scrolls found at Qumran in 1947.

Montefiore[9] considers another important point from ancient Jewish tradition. The Messiah–Haggadah[10] discusses the heptad in which the Son of David will come and states that in the fifth year "the Star shall shine forth from the East and this is the Star of the Messiah . . . and at the close of the seventh the Messiah is to be expected". This and other statements in the Midrashim in Jellenek's collection[11] indicate that a Star in the East was to appear two years before the birth of the Messiah. This ties in rather interestingly with Matthew II, 16 "Then Herod, when he saw that he was mocked of the wise men, was exceeding wrath and sent forth, and slew all the children that were in Bethlehem, and in all the coasts thereof, from 2 years old and under, according to the time which he had diligently enquired of the wise men". This "time" was obviously not the time of Christ's birth, which the Magi did not know when they first visited Herod, but was the time of the first appearance of the star. Using the triple conjunction hypothesis this occurred on 29 May 7 BC. Taking the old tradition that this would occur 2 years before the birth of the Messiah, Herod concluded that Jesus could have been born at any time between 29 May 7 BC and 29 May 5 BC and killed all the children of 2 years and under to be on the safe side.

Palmer[11] is perfectly correct in stating that the Magi could (like Lawrence of Arabia) have travelled from Babylonia to Jerusalem in 10 to 14 days and this must be taken as the lower limit for the journey time. The four months' journey time was mentioned in ref. 1 because this was the time taken by the Israelites when they were lead across the desert by Jehova on their return from captivity in Babylon. There is also a strong possibility that the Magi did not travel directly across the desert but took the easier but more distant route around the fertile crescent (Babylon, Mari, Carchemish, Ugarit, Byblos, Jerusalem—see Keller[12]).

Finally, a philological point[13]. Matthew uses the word star (αστηρ) four times (Matthew II, 2, 7, 9, 10) and each time it is used in the singular. Now αστηρ is not a word that is spelt the same way in both its singular and plural forms (the plural is αστερες), nor is it a collective noun (αστρον being a constellation), so it should not have been used to refer to more than one star. Second, if the 'star' of Bethlehem was a conjunction of two planets why didn't Matthew refer to 'planets' (πλανης αστηρ, a wandering star) and not stars? In answering these two points it must be remembered that Matthew's native language was not Greek, and also that Greek of the New Testament is not the classical Greek but the κοινη or popular everyday Greek, so words might not have been used in their strictly classical sense. Also Matthew, like other New Testament authors adhered in style and usage to the language of the Old Testament. 'Star' appears 24 times in the Old Testament, the word planet not appearing at all. So Matthew might just have used the better known expression.

The Matthean use of the singular was thought by Gerhardt[14] to refer simply to one planet in 7 BC, Saturn, the planet that was in conjunction with Jupiter. Saturn was "the protector of the Jews and reigned over their religion"—it was thus their star and the symbol of their Messiah.

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3 Edwards, O. *A New Chronology of the Gospels* (Floris, London, 1972).
4 Josephus, F. *Antiquities of the Jews* (trans. Thackeray, H. & Marcus, R. London, 1930)
5 Addey, J *Astrological J* 1 (July, 1959).
6 Kestranek, W. *American Astrology*, April, 1954.
7 *The Urantia Book*, 1351 5 (Urantia Foundation, Chicago, 1957).
8 Steiner, R. *Sun Wisdom, Moon Religion, Sun Religion* (London, 1950)
9 Montefiore, H. W *Novem Testementum* II, 211 (E J Brill, Leiden, 1960).
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12 Keller, W *The Bible as History* (Hodder and Stoughton, London, 1956).
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14 Gerhardt, O. *Des Stern des Messias* (1922).

Sodium and chloride in rainwater

PATERSON and Scorer[1] have suggested that methods used in rainwater analysis for Na^+ and Cl^- may give incorrect results and the increase in Na/Cl ratio reported by Hutton and Leslie[2] for inland samples collected in Victoria (Australia) relative to coastal samples may be an artefact. These suggestions are not acceptable.

Contamination of rainwater samples by Na is always possible and must be prevented as far as is practicable. In some Victorian samples Na was recorded at 0.2 μmol per 10^{-4} m^3 with the limit of detection being 0.1 and thus Na contamination should not be significant in samples reported as having 2 μmol per 10^{-4} m^3 or more. The limit of detection of Cl was 0.5 μmol per 10^{-4} m^3 and this limit imposes an artificial restriction on the Na/Cl ratio in dilute samples. Seawater with a Na/Cl ionic ratio of 0.858 when diluted to a Cl concentration of 3 μmol per 10^{-4} m^3 will have a ratio of 1 due to rounding to the nearest whole number and the value of R_s of 1.8 expected by Paterson and Scorer[1] will not be observed in at least 12 of the Victorian samples in their Fig. 3, it will be 1.5.

Nevertheless, there is enough evidence in the Victorian data to conclude that terrestrial sources contribute significant Na particularly at inland stations in summer and autumn. Hutton and Leslie[2] in their Fig. 3 show seasonal variations at six stations from coast to 320 km inland and in 14 out of the 17 sets of data the Na/Cl ratio in autumn is equal to or greater than the ratio in winter and in 9 out of the 11 sets of data the ratio in spring is equal to or less than the ratio in summer. Furthermore, the data in Table 1 give the following regression for 1955

$$\mathrm{Na/Cl} = 0.89 + 0.0022 \times d$$

($r^2 = 0.529$, $n = 24$) and for 1956

$$\mathrm{Na/Cl} = 0.86 + 0.0008 \times d$$

($r^2 = 0.454$, $n = 8$). (d = distance (km) from ocean in south-west direction). In both years at zero distance the value of the Na/Cl ratio is close to 0.86, the seawater ratio, and that in 1956, the wetter year for places usually receiving less than 625 mm of rain, the ratio changes less with distance.

It may be that the Eskdalemuir and Lerwick data (Fig. 2 in ref. 1) are not in error and the Wraymires site may be influenced by abnormal local factors.

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[1] Paterson, M. P. & Scorer, R. S. *Nature* **254,** 491–495 (1975).

[2] Hutton, J. T. & Leslie, T. I. *Aust. J. Agric. Res.* **9,** 492–507 (1958).

PATERSON REPLIES—Hutton has missed the point of our discussion[1] of the published data on ionic ratios of sodium and chloride in precipitation. Our analysis of data from a number of studies, most of them connected with the European Air Chemistry Network, found that current theories on segregation of these elements after originating in sea salt aerosol, were based on uncorroborated data. When independent results allowed consistency to be checked, no support was found for any real deviation from the seawater ratio of Cl/Na which is 1.80 by weight. If chlorine in some form is driven off the sea salt through chemical or photochemical action, it seems to be brought back again in any cloud droplets which form on the aerosol, as would be expected from the high solubility of the Cl_2 or HCl which are the postulated gaseous forms. The Australian data of Hutton and Leslie[2] was introduced into the discussion because their finding of a deviation from the sea salt ratio, which may be an important result as it has been found in the rather different environment of the dry and clear Australian atmosphere, also requires corroboration by an independent system of sample collection and analysis. Hutton's continuing failure to come to grips with this consideration is indicated by his final statement, made without supporting argument, that "the Eskdalemuir and Lerwick data (in ref. 1) (may not be) in error and the Wraymires site may be influenced by abnormal local factors."

Hutton's claim (refs 2, 3) that much of the sodium and chloride in inland samples arises from terrestrial sources is supported by a considerable weight of evidence, including the reanalysis of Hutton's Mildura data[4] by Paterson[5] which indicates that both elements are collected by that rain gauge at a rate which is effectively independent of the amount of rain during the month. This suggests that much of it has arrived as a dry deposit of aerosol, in quantities substantially greater than those found at a similar distance inland in Europe and North America. The range of atmospheric transport of these particles remains an open question, especially in view of Bigg's observation (personal communication) that extensive sampling from aircraft over wide areas of inland Australia has revealed very low concentrations of particles from any natural source. Perhaps the explanation is to be found in Dulhunty's observation[6] of the intermittent but intense dry storms which raise material from the dry salt lakes in brief episodes. Galbally (personal communication) has reported sightings from the air of 'salt devils' raised by willy willies crossing the dry lake beds.

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[1] Paterson, M. P. & Scorer, R. S. *Nature* **254,** 491 (1975).

[2] Hutton, J. T. & Leslie, T. I. *Aust. J. Agric. Res.* **9,** 492 (1958).

[3] Hutton, J. T. *Nature.*

[4] Hutton, J. T. *Arid Zone Research* **11,** 285 (UNESCO, Paris, 1958).

[5] Paterson, M. P. thesis, London Univ. (1975).

[6] Dulhunty, R. *The Spell of Lake Eyre,* 5 (Lowden Publishing, Kilmore, 1975).

Habitat values and endemicity in the vanishing rain forests of Sri Lanka

In our paper[1] the following scientific names were misspelled due to proof reading by committee. The correct spellings are *Nannophrys guentheri, Ramanella obscura, Cophotis ceylanica, Lyriocephalus scutatus, Otocryptis wiegmanni, Geckoella triedrus, S. taprobanensis, S. diegnani, Nessia burtoni* and *A. hickanala.*

Table 2 in the same paper was based on inaccurate estimates of the areas of the Regions. Our figures were obtained from the Department of Agriculture of Sri Lanka and were too large by a factor of about 1.4. We have prepared a more accurate rendition of the table using the areas in Kahawita[2] (Table 1), but since the ratios of the areas of the four regions are hardly affected the ratios of the habitat values and our conclusions are conserved.

We thank Dr H. Crusz at the Department of Zoology, University of Sri Lanka, Peradeniya, Sri Lanka, for pointing out the errors.

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[1] Senanayake, F. R., Soulé, M. & Senner, J. W. *Nature* **265,** 351–354 (1977).

[2] Kahawita, R. *Proc. Ceyl. Ass. Adv. Sci.* 8th Annual Session, II, 63–85 (1953).

Table 1 Habitat value data and indices for the amphibans, lizards and birds of Sri Lanka

	Area	Endemic Amphibians			Endemic lizards			Endemic birds			Totals		
Region	(km²)	No.	V_i	V'_i	No.	V_i	V'_i	No.	V_i	V'_i	No.	V_i	V'_i
A	43,302	0	0	0	5	0.12	0.12	3	0.07	0.05	8	0.19	0.17
B	10,742	5	0.47	0.39	7	0.65	0.65	11	1.02	0.43	22	2.14	1.47
C	7,851	7	0.89	0.71	10	1.27	1.27	18	2.29	1.09	35	4.45	3.07
D	2,873	8	2.78	2.27	9	3.13	3.13	18	6.27	2.34	35	12.18	7.74

reviews

Physical space and time

D. J. Raine

Space and Time in the Modern Universe. By P. C. W. Davies. Pp. viii+232. (Cambridge University: Cambridge, London and New York, 1977.) Hardback £6.50; paperback £2.95.

THERE was a time when the sins of the world would be explained through Quantum Theory and excused by Relativity. But the relevance of science to society is not so crude, and in due course Heisenberg and Einstein and their friends were returned to Academe. Nor is the relevance of science to be measured solely in terms of the subsequent military and technological applications, for the General Theory of Relativity at least—in some contrast to the Special Theory—has as yet no such applicability. This much it shares with the Copernican theory. That new vision has not changed one bit the motion of the Sun, or helped us to burn its light. Yet, as the contemporary Church understood well that it would, in shaping the way we see ourselves in relation to the World, the Copernican view has influenced our lives more than any display of military genius or noble act of statesmanship. And General Relativity, as part of our newly forming picture of the space time of the Universe may yet prove to have a similar relevance.

In *Space and Time in the Modern Universe,* Dr Davies is concerned to communicate, in a basically non-mathematical form, the status of our present understanding of physical space and time. He takes the reader from the mathematical idea of the continuum through Newtonian mechanics to electromagnetic theory and the space time of Special Relativity; from Newtonian gravity to the space time of General Relativity; from the microscopic world of the quantum to the Universe as a whole. Among the unresolved problems to which we are introduced are the reconciliation of the time symmetry of physical laws and the time asymmetry of natural processes; the reconciliation of Quantum Theory and General Relativity, still far from understood despite the progress which has been made in the quantum mechanics of black holes; and the reconciliation of the successes of General Relativity with the fact that it seems, in the singularity theorems, to predict its own inadequacy.

To encompass this vast range, Dr Davies adopts what one might call the synoptic approach. The danger of this style is the ease with which it becomes a mere recital of the table of contents of the book of physical thought. So wide-ranging is the discussion that the author's skill in making a point with a deftly chosen analogy or a succinct diagram does not always enable him to escape this pitfall. Thus, quasars are no more than "strange objects", spontaneous emission is not stimulated as in lasers, and escape velocity is *"escape velocity"*.

In selecting concepts for further discussion—and clearly considerable selection is required—there is a wide variation in the demands made on the reader. For example, he is expected to understand that higher frequency photons have higher energies (mentioned in passing in a discussion of radiation pressure); or consider ". . . the presence of electrically conducting materials will modify the fluctuations of photons ('particles' of electromagnetic radiation)" which, albeit for the second time, contains the only definition of photons given and for which more than a passing acquaintance with physics is required. On the other hand, the book starts by distinguishing between space and "outer space"; great care is taken to explain that the Solar System consists of the Sun and nine planets; and can anyone who does not already know what an element is, be expected to find it clear that a hot gas emits X rays?

These might be thought to be minor blemishes from which not even a masterpiece would be entirely free. I feel, however, that they are more likely to be symptomatic of an underlying unresolved pedagogic problem. For example, if it is emphasised that Special Relativity is a macroscopic theory which does not apply in the atomic domain, how is anyone without a high degree of physical maturity to understand that it is by incorporating this same relativity into the atomic description that Dirac discovered anti-matter? And compare the beautiful construction and dramatic effect of the chapter on time asymmetry with the visible discomfort at the need to explain the 'extraordinary' Quantum Theory *ab initio* in a few pages—and the almost audible sigh of relief on returning to relativity.

To be fair, it is indeed clearly stated that the book is addressed to those who already know enough of the subject to have asked themselves what might happen before the 'big bang' or at the centre of a black hole. Yet in the writing of the book, it seems that at times Dr Davies must have felt he might reach a wider audience. Certainly the results are of such importance that one can understand the desire to communicate them beyond the undergraduate physicist. But the problems are so difficult that it is probably impossible to satisfy entirely the whole of this wider audience in a single book.

In the final chapter of the book, Dr Davies turns to Man and the Universe. Here what matters is not so much the details of what has gone before but rather that it provides a reasonably coherent view of a Universe evolving. If the Universe is infinite and if the probability of the emergence of life is greater than zero, then there are infinitely many beginnings to Civilisation. It may be the destiny of some of these to evolve, to understand and perhaps to control their World. We are too young to know. If only a physicist would claim that the physical basis of life is now understood—and if Dr Davies is too optimistic in claiming that we already understand the evolution of structure in the Universe—nevertheless, such an understanding certainly seems to be emerging. And if we look outwards from ourselves to view this new Universe, this puts a unique complexion on our lives. What is important is that as many people as possible should be brought to know this view. □

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Intermediate astronomy

Astronomy: Principles and Practice. Pp. 341. *Astronomy: Structure of the Universe.* Pp. 283. By A. E. Roy and D. Clarke. (Adam Hilger: Bristol, UK, 1977.) Hardback £10; paperback £7.50 each volume.

ASTRONOMY owes its educational value to a combination of separate factors: historical and literary associations, a wealth of comparatively easily understood applications of the scientific method, an increasingly significant relationship with the environment as revealed by modern space research, and its fundamental role in exploring what sort of Universe we live in. As Plato said, astronomy is good for the soul, and increasing recognition of this fact has led in recent years to the production of a large number of lively and beautifully illustrated books directed at 'liberal arts' students and bright youngsters, often making little or no use of formal mathematics; a few of these have been reviewed in *Nature* (**266**, 102, 3 March, 1977) by D. W. Hughes. For those with the mathematical equipment of a first-year honours student in physical sciences, an admirable compact survey of the whole field is provided by A. Unsöld's *New Cosmos* (of which an English version of the second edition is currently in press), whereas numerous other books deal with particular aspects in more detail.

The present two volumes by Roy and Clarke aim at an intermediate level. They are based on university and extramural lectures given by the authors at Glasgow over a number of years, and contain essentially the material of the first-year university course attended by beginning science students (who study a range of subjects at this stage) and a minority of arts students wishing to gain some knowledge of the workings of science and the scientific method. The first volume, beginning with naked-eye phenomena and a brief description of the observables, proceeds in classic Glaswegian tradition to give a thorough grounding in elementary spherical trigonometry, positional astronomy and celestial mechanics, including useful topical references to Megalithic observatories and space-probe orbits. A short introduction to atomic and radiation physics leads on to the second major section discussing optics, optical and radio telescopes, detectors and instruments, with a final chapter describing a number of practical projects, mostly concerned with positional astronomy but with some optics and spectroscopy as well, that involve very simple equipment. The second volume describes the objects and phenomena of astronomical research: members of the Solar System, tides and eclipses, stellar magnitudes, colours and spectroscopy, variable and binary stars, stellar atmospheres, interiors and evolution, stellar motions, nebulae, galactic structure, galaxies, cosmology, cosmogony and the question of extraterrestrial life.

Admirably illustrated and neatly packaged into 40 separate chapters with exercises, the two volumes are very successful in giving a clear exposition and in driving points home. The treatment and content, however, are not very well adapted to the needs of either honours students or liberal arts students, nor to those of bright youngsters. The mathematical approach is distinctly old-fashioned, with spherical triangles solved in remorseless detail (including logarithms of cosines written out in full), celestial mechanics without benefit of vector analysis, and trivial relationships involving stellar magnitudes spelled out at length.

The scope of the second volume is severely limited: to take just two examples, there is no mention of curves of growth or opacity in stellar atmospheres, and in the section on cosmology, General Relativity is merely mentioned as satisfying the classical observational tests without any account of the Principle of Equivalence or of any other idea connected with it, so that the notion of the curvature of space then comes right out of the blue. Thus the present book gives considerably less information on astrophysical topics than does the largely non-mathematical treatment of the entire field by G. O. Abell in *Exploration of the Universe*. The practical outlook, and the clarity and thoroughness of treatment (up to the chosen level) of those topics that have been selected for coverage, will, however, make this book a useful work of reference, especially for ambitious amateurs and for teachers of extramural courses.

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Viruses and cell surface changes

Cell Surface Reviews. Volume 2: Virus Infection and the Cell Surface. Edited by G. Poste and G. L. Nicolson. Pp. 342. (North-Holland: Amsterdam, New York and Oxford, 1977.) $55.95; Dfl.137.

THE nature of surface changes induced by interactions between viruses and cell membranes is currently a topic of considerable interest to many virologists and cellular immunologists. This follows the realisation that cell-mediated immunity in virus diseases reflects surface monitoring of virally modified cells by re-circulating thymus-derived lymphocytes (T cells). Both viral and host components are apparently involved in T cell recognition. The evidence that has led to this concept is reviewed here by Blanden, Pang and Dunlop, and Burns and Allison. The area is, however, moving quickly and this volume is rather tardy, so some of the ideas expressed now have little currency.

The subject has not always been so fashionable. In fact, most of the virologists writing for this book are either unaware of, or have chosen to ignore, the trendy speculations of the cellular immunologists. This is somewhat of a relief. The result is a series of detailed reviews of established knowledge in areas that seem, in the main, to be relatively stable. The topics covered range through interactions of viruses with tissues (Smith, Weiner and Johnson), nucleated cells (Hosaka and Shimizu, Burns and Allison), erythrocytes (Bächi, Deas, and Howe) and model membrane systems (Tiffany). A thorough account of the structure and assembly of viral envelopes is provided by Rott and Klenk. Those considering the problem of immunological recognition of virally modified cells may benefit considerably from exposure to this information.

If I were to recommend, however, that a library acquire this very expensive volume, it is the article by Lindenmann that would induce me to do so. Lindenmann considers the various steps that led to general acceptance of the idea that a host-coded component, a carbohydrate moiety, is incorporated in budding influenza viruses. He uses this as a format to discuss both how science is done, and how credit is given (or denied) for what is done. The article is extremely readable, being written with some humour and considerable insight. This is recommended reading for students of virology, immunology and of the philosophy of science.

The book is well produced. Unfortunately, it has one major defect for those of us who read everything backwards. Individual articles are only identified at the beginning of each chapter. Printing of the authors names and/or a short title at the top of facing pages would make future volumes much more accessible for rapid reference.

P. C. Doherty

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Mammalian cell membranes

Mammalian Cell Membranes. Vol. 4: Membranes and Cellular Functions. Pp. 262. Vol. 5: Responses of Plasma Membranes. Pp. 260. Edited by G. A. Jamieson and D. M. Robinson. (Butterworths: London and Boston, Massachusetts, 1977.) £18 each volume.

WITH these books, Jamieson and Robinson close the circle of their edited series on the structure and function of mammalian cell membranes. Volume 4 is concerned with ultrastructural, biochemical and physiological aspects. In the first chapter Deamer provides a brief account of freeze-etch images and an interpretation of intramembraneous particles. This is a useful companion to later discussions of the permeability of membranes by De Pont and Bonting, and of membrane action potentials by Carpenter.

The bulk of Volume 4 is concerned with functional aspects of cell surface membranes and receptor theory. The antigenicity of cell surfaces is well covered by Weiss and Klavins—in particular, the section on the structural relationships between CEA and blood group substances—although I was startled to see references to Feizi *et al.* (1900) on pp. 74 and 95 in view of the sensationally youthful appearance of Dr Ten Feizi of the Northwick Park Clinical Research Centre.

Surface immunoglobulins and the controversy of the T-cell receptor are dealt with admirably by Kern, and Edwards provides a succinct account of current thinking in cell adhesion. Ashwell summarises the present belief of many concerning the role of surface carbohydrates in recognition phenomena, including some of those appearing in Edwards' chapter. In addition to describing briefly his own classical experiments, Ashwell reminds us of the pioneering and still provocative work of the Gasics in the related area of cell surface carbohydrates and host responses to tumours.

Volume 5 is concerned with the way in which the external environment affects the cell surface membrane, and *vice versa.* The environment in the most fundamental sense includes the effects of hydrostatic pressure (Zimmerman and Zimmerman), water structure (Meryman) and radiations (Patrick) on membranes. At the other extreme of specificity, Birnbaumer *et al.* make interesting sense of the huge literature on receptor coupling with adenyl cyclase, and on the regulation of the coupling by nucleotides. Strangely enough, the action of cholera toxin on membranes is not mentioned as a 'model' of hormone interactions with target cells.

In many biological processes, remodelling of cell surface membranes must occur. Several examples are considered in this volume, including endocytosis by Stockem, membrane fusion by Barski, membrane changes accompanying normal cell growth by Knox and Pasternak, and the senescent process by Robert. The best characterised of all remodelling processes, accompanying viral infection, is excellently treated by Klenk. The basic mechanisms by which membrane remodelling occurs is still a matter of speculation, although Scanlin and Glick clearly define the biochemical principles and dynamic nature of membrane turnover and discuss the concept of membrane flow.

I have now had the opportunity to read or browse through all five books of the series on *Mammalian Cell Membranes* and no doubt shall continue to profit from the immense amount of information therein for many years. The editors are to be congratulated on the success of their project, and they and their colleagues deserve our gratitude for providing a solid platform from which to view the rapidly changing membrane scene.

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Strange compendium

Handbook of Unusual Natural Phenomena. By William R. Corliss. Pp. 542. (The Sourcebook Project; Glen Arm, Maryland, 1977.) $14.95.

WILLIAM CORLISS has been producing his 'sourcebooks' of such strange phenomena as ball lightning, plagues of frogs and miscellaneous phenomena associated with earthquakes since 1974. In the past, these have taken the form of loose-leaf folders containing synoptical accounts of observations reported in a wide variety of journals (with *Nature* prominent among them) and over a long period (back into the nineteenth century). The present 'handbook' presents the mixture as before but in more substantial and permanent form, as a bound volume of more than 500 pages, full of fascinating morsels.

The entries range in length from a couple of lines to a page or more plus diagrams, and cover in this volume phenomena grouped together as luminous phenomena, optical and radio anomalies, unusual weather phenomena, mysterious natural sounds, strange phenomena of earthquakes, phenomena of the hydrosphere and falling material. For anyone suffering from the delusion that science has reached a comforting state of explaining just about everything, the book may provide a few shocks; for the less sanguine among us, here is a delightful source of bed-time reading, full of anecdotes with which to amaze and amuse colleagues the next day. And, who knows, by reviving interest in such curiosities as the shower of sand eels reported by A. Meek in *Nature* (**102,** 46; 1918), Corliss may well encourage the development of an improved scientific understanding of some unusual natural phenomena.

John Gribbin

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Beat the machine

Advances in Computer Chess. Edited by M. R. B. Clarke. Pp. 118. (Edinburgh University: Edinburgh, 1977.) £3.50.

IT is now many years since the electronic computer greatly exceeded the human brain in terms of speed and accuracy in calculation. But even in applications to well defined, finite games, such as chess, where this quality is at a premium, computers cannot match the performance of the more able human players.

This human advantage can largely be attributed to the possession of an ill-defined capability known as 'common sense', a concept unknown to computers. In the situation of the chess game, its activities are manifested in the human's ability to reject most of the moves available to him as unworthy of analysis, and to analyse only those which are 'sensible'.

This book, consisting of a set of articles from a symposium held in Oxford in 1975, deals with the problem of programming 'common sense' into a computer. In the chess context, this amounts principally to techniques of search reduction at the tactical level, and rudimentary 'planning' at the strategic level. A short article on the mathematical theory of search techniques, and several examples of the success (or lack of it) of practical programmes, complete a collection which should be worthwhile reading for those interested in the problems of machine intelligence.

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Narrow gap semiconductors

Semimetals and Narrow-Bandgap Semiconductors. By D. R. Lovett. Pp. viii+256. (Pion: London, 1977.) £8.50; $17.50.

AT FIRST GLANCE this book has a pleasing appearance with many clear diagrams and graphs, and a more detailed examination has convinced me that the breadth and depth of coverage should make it a very useful book for those entering the field of electrical properties and applications of narrow gap semiconductors.

The book is basically in two parts. In the first part, there is an excellent and concise introduction to phase diagrams and crystal growing techniques, clearly outlining the many pitfalls and problems which would confront anyone trying to prepare pure and homogeneous materials. The chapter on crystal symmetry and band properties is probably the weakest part of the book, for it is so concise as to be practically of no help to anyone except those already initiated in the art. The electrical properties is well treated and is somewhat similar to Putley's well-known book *Hall Effect and Related Phenomena.* There is also a chapter briefly reviewing narrow band gap devices.

The real interest of the author is expressed in the second half of the book, dealing with the much more detailed aspects of particular materials, ranging from bismuth–antimony alloys to selenides, tellurides and arsenides. This latter part of the book would certainly be of very considerable use as a source of information for both the researcher as well as those interested in the application of narrow band semiconductors.

At £8.50 this book is certainly value for money for those on the periphery of this field.

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Analysing pure substances

Spectrochemical Analysis of Pure Substances. Edited by Kh. I. Zil' berstein. Translated by J. H. Dixon. Pp. 435. (Plenum: London and New York, 1977) £27.

THIS English translation of the (1971) Russian edition, stated by the publishers to be thoroughly revised and up-to-date, contains 1,497 references, over half to Russian work, but less than ten after 1971. A vast amount of information on trace analysis is given, most of it in an uncritical review form of Russian and Eastern European work.

In Part I, the first three chapters give excellent, but over-lengthy and unbalanced, sections on evaluating limits of detection, and their improvement by optimising parameters of spectral resolution. Reference to spectrographic equipment by symbol only would have been of more value if a table had been given listing their main features such as focal length. In chapter 4 a long description is given of arc sources which, although biased towards Russian work, describes in detail the various distribution parameters across electrode gaps of d. c. and a. c. arcs, with anode and cathode excitation, and the resulting line-to-background ratios. Techniques for introducing samples into the arc are reviewed and methods of stabilisation, such as the use of external magnetic fields and various forms of constricted arcs are discussed. Other sources are covered in less detail, but the glow discharge is noticeably absent.

Part 2 describes preliminary concentration methods, starting with a rather cumbersome mathematical section on the expression of the concentration factor, followed by more useful sections on concentration by volatilisation, sublimation, crystallisation, zone melting, solvent extraction, ion exchange, coprecipitation and electrodeposition.

Part 3 deals with practical problems including sampling, grinding, laboratory design, purification of reagents and carbon electrodes, and more briefly with the preparation of synthetic standards. The final section, on practical methods of analysing pure substances, is a ten-page list of sample types, noting the trace elements determined, and giving references in the bibliography.

The contents of the book and its translation are excellent. It is full of valuable information, but this can only be found by thorough study, as the index is totally inadequate. I can foresee two types of reader: the spectroscopist, who may obtain some new lines and those who wish to be made aware of Eastern-European work. Both would be justified in purchasing this book. Readers should note, however, that some of the Russian claims to priority are unjustified according to the record of world literature.

T. S. West

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Endocrinology yearbook

The Year in Endocrinology, 1975–1976. Edited by Sidney H. Ingbar. Pp. xviii+327. (Plenum Medical: New York and London, 1976.) $27.

'YEARBOOKS' come in many shapes and sizes. Probably the commonest format is the upgraded textbook in which a series of chapters, whose headings embrace non-selectively the whole of the subject under consideration, seek to convey to the reader the principal advances which have taken place since the previous edition. Often, each chapter will consist of summaries of key papers accompanied by editorial comment. The contrary approach is the presentation of a series of highly selective reviews by eminent authorities, usually with a heavy bias towards the work of their own groups. The present volume falls somewhere between the two. Nine chapters by individual authors review the principal endocrine systems except those of the foeto-placental unit and the gut. Most are comprehensive, but there are occasional notable exclusions: for example, the section on neuroendocrinology makes no mention of the posterior pituitary gland, whereas another concentrates on calcitonin and ignores parathyroid hormone. Finally, there are two special essays on ectopic hormone secretion and the mechanisms of steroid hormone action.

The general standard of the individual chapters is high and as a review of the state of the art for readers in 1977 there is no doubt that this is a useful book. The transatlantic flavour, however, is noticeable by the absence of comment on important therapeutic advances. For example, the use of ergot derivatives in the treatment of hyperprolactinaemia is dimissed in a few lines and a single minor reference. This is surprising since the use of Bromocriptine is probably the single most important development in endocrine management of the 1970s, and the bulk of the significant papers on this subject appeared in the time period in which this book purports to cover. Far more space is devoted to the use of the gonadotrophin-releasing hormone, despite the fact that it is unlikely this material will ever be of great practical value. These comments apart, the book can be commended to the practising endocrinologist.

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Recent scientific and technical books

Biology

JOHNSON, Martin H. (edited by). Development in Mammals, Vol. 1. Pp.vii+390. ISBN-0-7204-0631-5. (Amsterdam, New York and Oxford: North-Holland Publishing Company, 1977.) np.

JOHNSON, Martin (edited by). Development in Mammals, Vol. 2. Pp.vii+241. ISBN-0-7204-0646-3. (Amsterdam, New York and Oxford: North-Holland Publishing Company, 1977.) Dfl. 72; $29.50.

JUNGREIS, Arthur M., **HODGES**, Thomas K., **KLEINZELLER**, Arnost, and **SCHULTZ**, Stanley G. (edited by). Water Relations in Membrane Transport in Plants and Animals. Pp.xiv+393. ISBN-0-12-392050-7. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) $118; £12.80.

KAHRIG, Erwin, und **BESSERDICH**, Hartmut. Dissipative Strukturen: Eine Einführung in den Problemkreis und die Erscheinungsformen. (Fortschritte der Experimentellen und Theoretischen Biophysik, Band 21.) Pp.116. (Leipzig: Veb Georg Thieme, 1977.) DDR 29 Mark.

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KLUGE, Arnols G. In collaboration with **FRYE**, B. E. *et al.* Chordate Structure and Function. Second edition. Pp.x+628. ISBN-0-02-364800-7. (New York: Macmillan Publishing Co., Inc.; London: Collier Macmillan Publishers, 1977.) £12.

KORN, Edward D. (edited by). Methods in Membrane Biology, Vol. 8. Pp.xix+368. ISBN-0-306-36808-0. (v. 8). (New York and London: Plenum Press, 1977.) $39.50.

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LAGLER, Karl F., **BARDACH**, John E., **MILLER**, Robert M., and **PASSINO**, Dora R. May. Ichthyology. Second edition. Pp.xv+506. ISBN-0-471-51166-8. (New York and London: John Wiley and Sons, 1977.) £13.50; $23.50.

LOVTRUP, Søren. The Phylogeny of Vertebrata. Pp. xii+330. ISBN-0-471-99412-X. (London and New York: Wiley-Interscience, John Wiley and Sons, 1977.) £15; $29.50.

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COOLS, A. R., **LOHMAN**, A. H. M., and **VAN DEN BERCKEN**, J. H. L. (edited by). Psychobiology of the Striatum. Pp.xii+197. ISBN-0-7204-0633-1. (Amsterdam, New York and Oxford: North-Holland Publishing Company, 1977.) Dfl. 73; $29.95.

COULSTON, Frederick, and **KORTE**, Freidhelm (edited by). Environmental Quality and Safety: Global Aspects of Chemistry, Toxicology and Technology as Applied to the Environment, Vol. 5. Pp.259. ISBN-0-12227005-3. (Stuttgart: Georg Thieme Verlag; New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1976.) $18.50; £9.60.

CROSIGNANI, P. G., and **ROBYN**, C. (edited by). Prolactin and Human Reproduction. (Proceedings of the Serono Symposia, Vol. 11.) Pp.vii+305. ISBN-0-12-198345-5. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £10.50; $20.50.

CUATRECASAS, P., and **GREAVES**, M. F. (edited by). Receptors and Recognition, Series A. Pp. viii+166. ISBN-0-412-14320-8. (London: Chapman and Hall; New York: Halstead Press, a Division of John Wiley and Sons, Inc., 1977.) Cased £10; Paperback £6.

CURRY, Stephen H. Drug Disposition and Pharmacokinetics, with a Consideration of Pharmacological and Clinical Relationships. Pp.xi+275. Second edition. ISBN-0-632-00476-2. (Oxford and London: Blackwell Scientific Publications, 1977. £6.25.

DALY, John. Cyclic Nucleotides in the Nervous System. Pp. xiv+401. ISBN-0-306-30971-8. (New York and London: Plenum Press, 1977.) $39.

DAY, Charles E., and **LEVY**, Robert S. (edited by). Low Density Lipoproteins. Pp.xx+445. ISBN-0-306-30934-3. (New York and London: Plenum Press, 1976.) $47.40.

DAY, Nooribi, and **GOOD**, Robert A. (edited by). Biological Amplification Systems in Immunology. (Comprehensive Immunology, Vol. 2.) Pp.xv+325. ISBN-0-306-33102-0. (New York and London: Plenum Medical Book Company, 1977.) np.

DRUCKER-COLIN, René R., and **McGAUGH**, James (edited by). Neurobiology of Sleep and Memory. (Proceedings of a Conference held in Mecixo City in March, 1975.) Pp.xiii+456. ISBN-0-12-222350-0. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) $19.50; £13.85.

DUNCAN, W. A. M., and **LEONARD**, B. J. (edited by). Clinical Toxicology. (Proceedings of the Meeting held at Edinburgh, June 1976.) (Proceedings of the European Society of Toxicology, Vol. XVIII, 1977.) Pp.ix+348. ISBN-0-444-15248-2. (Amsterdam and Oxford: Excerpta Medica, 1977. Distributed in the USA and Canada by Elsevier North-Holland, Inc., New York.) Dfl. 87.50; $35.75.

DUNCAN, Wm., and **NIAS**, A. H. W. Clinical Radiobiology. Pp.226. ISBN-0-443-01147-8. (Edinburgh, London and New York: Churchill Livingstone, 1977.) £9.50.

ELIAS, P. S., and **COHEN**, A. J. (edited by). Radiation Chemistry of Major Food Components: Its Relevance to the Assessment of the Wholesomeness of Irradiated Foods. Pp.xii+220. ISBN-0-444-41587-4. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl. 63; $25.75.

ELLINWOOD, Jr., Everett H. (edited by). Cocaine and Other Stimulants. (Advances in Behavioral Biology, Vol. 21.) Pp.x+721. ISBN-0-306-37921-X. (New York and London: Plenum Press, 1977.) $59.40.

EPSTEIN, T. Scarlett, and **JACKSON**, Darrell (edited by). The Feasibility of Fertility Planning: Micro Perspectives. Pp.viii+244. ISBN-0-08-021837-7. (Oxford and New York: Pergamon Press, 1977.) Hardcover £12.50; Flexicover £4.

ESSMAN, Walter B. (edited by). Serotonin in Health and Disease. Vol. 4: Clinical Correlates. Pp.444. ISBN-0-89335-003-6 (v. 4). (New York: S.P. Books Division of Spectrum Publications, Inc., 1977.) $37.50.

FERTILIZER USE AND PROTEIN PRODUCTION. (Pøoceedings of the 11th Coloquium of the International Potash Institute held in Rzonne-Bornholm, Denmark, 1975.) Pp.307. (Worblaufen-Bern: International Potash Institute, 1975.) Sw. fr. 28; $11.70.

THE FETUS AND BIRTH. (Ciba Foundation Symposium 47, New Series.) Pp.viii+481. ISBN-0-444-15255-5. (Amsterdam, Oxford and New York: Elsevier/Excepta Medica/ North-Holland, 1977.) Dfl. 98; $39.95.

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FRANKEL, R., and **GALUN**, E. Pollination Mechanisms, Reproduction and Plant Breeding. (Monographs on Theoretical and Applied Genetics, Vol. 2.) Pp.xi+281. ISBN-3-540-07934-3. (Berlin and New York: Springer-Verlag, 1977.) DM 60; $26.40.

FREINKEL, Norbert (edited by). The Year in Metabolism, 1975-1976. Pp.xxi+353. ISBN-0-306-32001-0. (New York and London: Plenum Medical Book Company, 1976.) $27.

FREY, R., **NAGEL**, E., and **SAFAR**, P. (edited by). Mobile Intensive Care Units: Advanced Emergency Care Delivery Systems. (Anaesthesiology and Resuscitation/Anaesthesiologie und Wiederbelebung/Anesthésiologie et Réanimation, Vol. 95.) Pp.xv+271. ISBN-3-540-07561-5. (Berlin and New York: Springer-Verlag, 1976.) DM 48; $19.70.

FRIGERIO, Alberto, and **GHISALBERTI**, Emilio L. (edited by). Mass Spectrometry in Drug Metabolism. Pp.xii+532. ISBN-0-306-31018-X. (New York and London: Plenum Press, 1977.) np.

GLICK, David, and **ROSENBAUM**, Robert M. (edited by). Techniques of Biochemical and Biophysical Morphology, Vol. 3. Pp.ix+214. ISBN-0-471-02219-5. (New York and London: Wiley-Interscience, a Division of John Wiley and Sons, Inc., 1977.) £17.25; $29.

GOLTERMAN, H. L. (edited by). Interactions Between Sediments and Fresh Water. (Proceedings of an International Symposium held at Amsterdam, The Netherlands, September 6-10, 1976.) Pp.473. ISBN-90-06193-563-6. (The Hague: Dr. W. Junk B.V. Publishers; Wageningen: Centre for Agricultural Publishing and Documentation, 1977.) Dutch guilders 65.

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GREATHEAD, D. J. (edited by). A Review of Biological Control in Western and Southern Europe. (Technical Communication No. 7.) Pp.182. ISBN-0-85198-369-3. (Farnham Royal, Slough: Commonwealth Agricultural Bureaux, 1976.) np.

GROTE, Jürgen, **RENEAU**, Daniel, and **THEWS**, Gerhard (edited by). Oxygen Transport to Tissue-II. (Advances in Experimental Medicine and Biology, Vol. 75.) Pp.xxiii+781. ISBN-0-306-39075-2. (New York and London: Plenum Press, 1976.) $59.40.

HAMILTON, Leonard W. Basic Limbic System Anatomy of the Rat. Pp.xvii+150. ISBN-0-306-30925-4. (New York and London: Plenum Press, 1976.) $23.40.

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HARDIN, Garrett, and **BADEN**, John (edited by). Managing the Commons. Pp.xii+294. ISBN-0-7167-0476-5. (San Francisco and Reading: W. H. Freeman and Company, 1977.) £5.10.

HARRISON, R. G., and de **BOER**, C. H. Sex and Infertility. (Monographs for Students of Medicine.) Pp.xii+122. ISBN-0-12-327850-3. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £2.80; $5.50.

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IMMUNOLOGY OF THE GUT. (Ciba Foundation Symposium, No. 46, New Series.) Pp.viii+376. ISBN-0-444-15246-6. (Amsterdam, Oxford and New York: Elsevier/Excerpta Medica/North-Holland, 1977.) Dfl. 70; $28.75.

IMPERATO, Pascal James. African Folk Medicine: Practices and Beliefs of the Bambara and Other Peoples. Pp.xvii+251. ISBN-0-912752-08-4. (Baltimore, Md.: York Press, Inc., 1977.) $16.

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JACKSON, I. J. Climate, Water and Agriculture in the Tropics. Pp.xii+248. ISBN-0-582-48529-0. (London and New York: Longman, 1977.) £3.75.

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KANE, Robert L. (edited by). The Behavioral Sciences and Preventive Medicine: Opprtunities and Dilemmas. (A Conference sponsored by the John E. Fogarty International Center for Advanced Study in the Health Sciences, and the Association of Teachers of Preventive Medicine, National Institutes of Health, Bethesda, Md., December 5 and 6, 1974.) Pp.xxvi+261. (Washington, DC: US Government Printing Office, 1977.) $6.70.

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KISSIN, Benjamin, and **BEGLEITER**, Henri (edited by). Treatment and Rehabilitation of the Chronic Alcoholic. (The Biology of Alcoholism, Vol. 5.) Pp.xxv+631. ISBN-0-306-37115-4 (v. 5). $47.40.

KLEIN, George, and **WEINHOUSE**, Sidney (edited by). Advances in Cancer Research, Vol. 25. Pp.ix+401. ISBN-0-12-006625-4. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) $29.50; £20.95.

KLINGMULLER, W. Genmanipulation und Gentherapie. Pp.viii+345. ISBN-3-540-07903-3. (Berlin and New York: Springer-Verlag, 1976.) DM 38; $15.60.

KNOWLES, John H. (edited by). Doing Better and Feeling Worse: Health in the United States. Pp.278. (New York: W. W. Norton and Company, 1977.) Cloth $9.95; Paper $3.95.

KRAMER, M., and **LAUTERBACH**, F. (edited by). Intestinal Permeation. Proceedings of the Fourth Workshop Conference Hoechst, Schloss Reisenburg, 19–22 October, 1975.) (Workshop Conferences Hoechst, Vol. 4.) Pp.xii+449. ISBN-0-444-15225-3. (Amsterdam and Oxford: Excerpta Medica, 1977. Distributed in USA and Canada by Elsevier North-Holland, Inc., New York.) Dfl. 160; $65.50.

KRAUSE, W. (edited by). Application of Vegetation Science to Grassland Husbandry. (Handbook of Vegetation Science, Part 13.) Pp.xx+535. ISBN-90-6193-194-0. (The Hague: Dr. W. Junk b.v. Publishers, 1977.) Dutch guilders 150.

LURIYA, E. A. Hematopoietic and Lymphoid Tissue in Culture. Translated from Russian by Basil Haigh. (Studies in Soviet Science.) Pp.xiii+181. ISBN-0-306-10934-4. (New York and London: Consultants Bureau a Division of Plenum Publishing Corporation 1977.) $42.

LAL, Harbans (edited by). Discriminative Stimulus Properties of Drugs. (Advances in Behavioral Biology, Vol. 22.) Pp.xii+239. ISBN-0-306-37922-8. (New York and London: Plenum Press, 1977.) $30.

LANCE, E. M., **MEDAWAR**, P. B., and **SIMPSON**, E. (edited by). An Introduction to Immunology. Pp.xii+158. ISBN-0-7045-0209-7. (London: Wildwood House, Ltd., 1977.) £4.95 net.

LAUFFER, Max A., **BANG**, Frederick B., **MARAMOROSCH**, Karl, and **SMITH**, Kenneth M. Advances in Virus Research, Vol. 21. Pp.vii+409. ISBN-0-12-039821-4. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) $29.50; £20.95.

LEE, Peter A., **PLOTNICK**, Leslie P., **KOWARSKI**, A. A., and **MIGEON**, Claude J. (edited by). Congenital Adrenal Hyperplasia. Pp.xxii+532. ISBN-0-8391-0974-1. (Baltimore and Tokyo: University Park Press; Lancaster: MTP Press, Ltd., 1977.) £26.95.

LOOR, F., and **ROELANTS**, G. E. (edited by). B and T Cells in Immune Recognition. Pp.xviii+504. ISBN-0-471-99438-3. (London and New York: Wiley-Interscience, John Wiley and Sons, 1977.) £18.50; $37.50.

MAKINODAN, Takashi, and **YUNIS**, Edmond (edited by). Immunology and Aging. (Comprehensive Immunology, Vol. 1.) Pp.xv+208. ISBN-0-306-33101-2 (New York and London: Plenum Medical Book Company, 1977.) $27.

MADDEN, J. S., **WALKER**, Robin, and **KENYON**, W. H. (edited by). Alcoholism and Drug Dependence: a Multidisciplinary Approach. Pp.xix+479. ISBN-0-306-31019-8. (New York and London: Plenum Press, 1977.) $47.40.

MARCHALONIS, John J. (edited by). The Lymphocyte: Structure and Function, Part 1. (Immunology Series, 5.) Pp.xi+369. ISBN-0-8247-6418-8. (New York and Basel: Marcel Dekker, Inc., 1977.) SFrs. 123.

MARTIN, Hubert, and **WORTHING**, Charles R. (edited by). Insecticide and Fungicide Handbook for Crop Protection. (Issued by the British Crop Protection Council.) Pp.xvi+427. Fifth edition. ISBN-0-632-00109-7. (Oxford and London: Blackwell Scientific Publications, 1976. Distributed In USA and Canada by J. B. Lippincott Company.) £12.50.

MARTINEZ-MALDONADO, Manuel (edited by). Methods in Pharmacology. Vol. 4A: Renal Pharmacology. Pp.x+387. ISBN-0-306-35264-8. (New York and London: Plenum Press, 1976.) $47.40.

MARZULLI, Francis N., and **MAIBACH**, Howard I. (edited by). Dermatotoxicology and Pharmacology. (Advances in Modern Toxicology, Vol. 4.) Pp.xviii+567. ISBN-0-470-99063-5. (Washington: Hemisphere Publishing Corporation, 1977. Distributed by Halsted Press, a Division of John Wiley and Sons, Inc., New York and Chichester.) £26; $44.

MASSRY, Shaul G. (edited by). Kidney in Systemic Diseases. (Contributions to Nephrology, Vol. 7.) Pp. viii+308. ISBN-3-8055-2445-5. (Basel, London and New York: S. Karger, 1977.) SFr/DM 98; $37.75.

MOHR, U., **SCHMAHL**, D. and **TOMATIS**, L. (edited by). **DAVID**, W. (technical editor). Air Pollution and Cancer in Man. (Proceedings of the Second Hanover International Carcinogenesis Meeting held in Hanover, 22-24 October, 1975.) Pp. xvii+331. ISBN-92-832-1116-2. (Lyon: International Agency for Research on Cancer, 1977. Distributed by WHO, Geneva.) S/Fr. 90; $35.

MORELL, Pierre (edited by). Myelin. Pp. xxii+531. ISBN-0-306-30965-3. (New York and London: Plenum Press, 1977.) $47.40.

MUNOZ, J. J. and **BERGMAN**, R. K. *Bordetella pertussis:* Immunological and Other Biological Activities. (Immunology Series, Vol. 4). Pp.235. ISBN-0-8247-6507-9. (New York and Basel: Marcel Dekker, Inc., 1977.) SFrs.82.

NAGAICH, B. B. (edited by). Recent Technology in Potato Improvement and Production. (Lectures delivered during the Summer Institute, held at the Central Potato Research Institute, Simla, from June 21 to July 20, 1976.) Pp.iv+310. (Simla: Central Potato Research Institute, Indian Council of Agricultural Research, 1977.) np.

NAIR, P. P., and **KRITCHEVSKY**, David (edited by). The Bile Acids: Chemistry, Physiology, and Metabolism. Vol. 3: Pathophysiology. Pp.xiii+229. ISBN-0-306-37133-2. (New York and London: Plenum Press, 1976.) $27.

OXFORD, J. S., **DRASAR**, F. A. and **WILLIAMS**, J. D. (edited by). Chemotherapy of Herpes Simplex Virus Infections. (Reprinted from Supplement A of the *Journal of Antimicrobial Chemotherapy*, Vol. 3, March 1977). Pp.vii+141. (London and New York: Academic Press, 1977.) £4.80; $9.35.

PAOLETTI, R., and **SHERRY**, S. (edited by). Thrombosis and Urokinase. (Proceedings of the Serono Conference, Vol. 9.) Pp.viii+257. ISBN-0-12-544960-7. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £8.20; $16.

PASQUALINI, Jorge E. (edited by). Receptors and Mechanism of Action of Steroid Hormones, Part 2. Modern Pharmacology-Toxicology: a Series of Monographs and Textbooks, Vol. 8.) Pp.ix+311-736. ISBN-0-8247-6440-4. (New York and Basel: Marcel Dekker, Inc., 1977.) SFrs.130.

PETTIT, George R. Biosynthetic Products for Cancer Chemotherapy, Vol. 1. Pp.xii+215. ISBN-0-306-37687-3 (v.1). (New York and London: Plenum Press, 1977.) $23.40.

PORTER, R. R. and **ADA**, G. L. (edited by). Contemporary Topics in Molecular Immunology, Vol. 6. Pp.xiii+252. ISBN-0-306-36106-X. (New York and London: Plenum Press, 1977.) $30.

PRASAD, M. R. N. and **KUMAR**, T. C. Anand (edited by). Use of Non-Human Primates in Biomedical Research. (International Symposium held in New Delhi, India, November 3-8, 1975.) Pp.426. (New Delhi: Indian National Science Academy, 1977.) np.

PRESTON, Thomas A. Coronary Artery Surgery: a Critical Review. Pp.xi+266. ISBN-0-89004-165-2. (New York: Raven Press, 1977.) $15.

PURINE AND PYRIMIDINE METABOLISM. (Ciba Foundation Symposium 48, New Series.) Pp.xi+369. ISBN-0-444-15256-3. (Amsterdam, Oxford and New York: Elsevier/Excerpta Medica/North-Holland, 1977.) Dfl.76; $30.95.

QURAISHI, M. Sayeed. Biochemical Insect Control: Its Impact on Economy, Environment, and Natural Selection. Pp.viii+280. ISBN-0-471-70275-7. (New York and London: Wiley/Interscience, John Wiley and Sons, 1977.) £14.25; $24.50.

RAJKA, E. and **KOROSSY**, S. (edited by). Immunological Aspects of Allergy and Allergic Diseases. Vol. 6: Antigen-Antibody Reactions in Different Organs. Pp.vii+343. ISBN-0-306-37756-X. (London and New York: Plenum Press; Budapest: Akadémiai Kiadó, 1976.) $42.

RATCLIFFE, Derek (edited by). A Nature Conservation Review: The Selection of Biological Sites of National Importance to Nature Conservation in Britain. Vol. 1: Pp.xvi+401. ISBN-0-521-21159-X. £35 net. Vol. 2: Pp.viii+320. ISBN-0-521-21403-3. £25 net. (Cambridge, London and New York: Cambridge University Press, 1977.)

RECHCIGL, Jr., M. (edited by). Nutrient Elements and Toxicants. (Comparative Animal Nutrition, Vol. 2.) Pp.x+210. ISBN-3-8055-2351-3. (Basle, London and New York: S. Karger, 1977.) SFr./DM.98; $37.75.

REID, Robert. My Children, My Children: Life Before and After Birth—an Account of Some Recent Developments. Pp.186. ISBN-0-563-12857-7. (London: British Broadcasting Corporation, 1977.) £3.50.

REINHOLD, L., **HARBORNE**, J. B., and **SWAIN**, T. (edited by). Progress in Phytochemistry, Vol. 4. Pp.289. ISBN-0-08-021004-X. (Oxford and New York: Pergamon Press, 1977.) £16.50.

REIVICH, Martin, **COBURN**, Ronald, **LAHIRI**, Sukhamay, and **CHANCE**, Britton. Tissue Hypoxia and Ischemia. (Advances in Experimental Medicine and Biology, Vol. 78.) Pp.xiv+385. ISBN-0-306-39078-7. (New York and London: Plenum Press, 1977.) $45.

announcements

On the Move

R. S. Barnes, Chief Scientist of the British Steel Corporation since 1975, and previously its Director of Research and Development, to Principal of Queen Elizabeth College, London University, on 1 April 1978.

K. G. Adiyodi, Profesor of Reproductive Physiology, Calicut University, India and Secretary, International Society for Invertebrate Reproduction, to Department of Zoology, Oxford University, South Parks Road, Oxford.

C. D. Bridges, Professor of Experimental Ophthalmology at New York University Medical Center, to Professor of Ophthalmology at the Baylor College of Medicine, Houston, Texas.

Professor Y. K. Levine from Department of Physical Chemistry, University of Leeds, to the Chair of Biophysics at the University of Utrecht.

Details of changes of department, sabbaticals, where leave will be taken and so on should be sent to On the Move; there is no charge for this service.

Appointments

Sir Ieuan Maddock, previously Chief Scientist at the Department of Industry, is to succeed Magnus Pyke as Secretary of the British Association for the Advancement of Science on 7 September 1977.

R. Frosch, previously a director of the Woods Hole Oceanographic Institution, to Head of NASA.

A. J. Lee, Director of Fisheries Research at Lowestoft for the Ministry of Agriculture, Fisheries and Food, to Controller of Fisheries Research and Development for Great Britain.

J. Black, Professor of Pharmacology at University College, London, is to join the Wellcome Research Laboratories at Beckenham, Kent, as Director of Therapeutic Research. He will lead the search for new chemical compounds and their testing for activity and safety with the aim of finding new medicines for the control of the functions of the body. He expects to be working at Beckenham full-time by 1 January, 1978.

A. C. Fabian to the newly-established Radcliffe Fellowship in Astronomy, to be based at the Cambridge Institute of Astronomy where his research will concentrate on X-ray astronomy and related optical studies.

W. D. M. Paton, Professor of Pharmacology at the University of Oxford is to succeed Sir J. McMichael as a trustee of the Wellcome Trust from 1 January 1978.

Awards

Dr J. E. Castle is the recipient of the Beilby Medal and Prize for 1977, the joint award of the Royal Institute of Chemistry, the Society of Chemical Industry and the Metals Society, for his work on the chemistry and metallurgy of surfaces, particularly studies of metallic corrosion by photoelectron spectroscopy.

Person to Person

Exchange furnished house in Richmond, Virginia, for similar in Cambridge, England from January 1978 to January 1979. Car exchange also. Contact Judith Bond, Department of Biochemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298, USA.

Exchange 2-bedroom apartment in Jerusalem for similar in Bethesda area, Maryland, for one year beginning September/October 1977. Contact D. BenEzra, National Institutes of Health, Bldg 10, Room 10D-09, Bethesda, Maryland 20014, USA.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

Meetings

12–14 September, **Conference on Scanning and Transmission Electron Microscopy,** Glasgow, UK (Institute of Physics, 47 Belgrave Square, London SW1, UK).

11–17 September, **VIth European Congress of Pathology,** London, UK (Conference Services Ltd, 43 Charles Street, London W1, UK).

13–15 September, **Particle Size Analysis Conference,** Salford, UK (D. Dollimore, Reader in Physical Chemistry, University of Salford, Salford, UK).

12–16 September, **3rd International Conference on Bioindicators and Landscape Deterioration,** near Prague, Czechoslovakia (Ustav krajinné ekologie CSAV (Ing. J. Spáleny CSc.) 252 43 Pruhonice, Czechoslovakia).

26–27 September, **Conference on Dynamic Small European Companies in the Health-Care Industry, and Why they are Successful,** Brussels (Robert S. First, Inc., Avenue Marnix, 19A, 1050 Brussels, Belgium).

12–14 October, **Working Group on Rehabilitation and Secondary Prevention in Patients with Acute Myocardial Infarction,** Valencia (WHO, Regional Office for Europe, 8 Schefigsvej, DK-2100 Copenhagen, Denmark).

11–13 August, **Annual Meeting of the American Society of Pharmacognosy,** Washington (L.R. Brady, Pharmacy, University of Washington, Seattle, Washington 98195.

1–2 September, **The 571st Meeting of The Biochemical Society,** Dublin (Meetings Officer, The Biochemical Society, 7 Warwick Court, London, UK).

4–8 September, **3rd International Conference on Microprobe Analysis in Biology and Medicine,** Munster, West Germany (P. Echlin, Department of Botany, University of Cambridge, Cambridge, UK).

5–9 September, **European Conference on Precise Electrical Measurement,** Brighton (Institute of Electrical Engineers, Savoy Place, London, UK).

6–9 September, **7th European Microwave Conference and Microwave 1977,** Copenhagen (MEPL, Temple House, 34–36 High Street, Sevenoaks, Kent, UK).

6–9 September, **Conference on Digital Processing of Signals in Communications,** Loughborough (Conference Secretariat, Institution of Electronic and Radio Engineers, 8–9 Bedford Square, London, UK).

6–8 September, **Conference on Scaling for Performance Prediction in Rotodynamic Machines,** London (The Institution of Mechanical Engineers, 1 Birdcage Walk, London, UK).

8 September, **Seminar on Corrosion and Fatigue in Offshore Installations,** London (The Institution of Mechanical Engineers, 1 Birdcage Walk, London, UK).

12–15 September, **7th European Conference on Solid State Devices Research,** Brighton (Meetings Officer, Institute of Physics, 47 Belgrave Square, London, UK).

8 September, **The Draughting and Preparation of an Iso Standard on Nuclear Quality Assurance,** London (The Institution of Mechanical Engineers, 1 Birdcage Walk, London, UK).

13 September, **Mini-Computers—Applications,** London (The Institution of Mechanical Engineers, 1 Birdcage Walk, London, UK).

13–15 September, **Conference on Heat and Fluid Flow in Water Reactor Safety,** London (The Institution of Mechanical Engineers, 1 Birdcage Walk, London, UK).

14–16 September, **3rd European Conference on Optical Fibre Communication,** Munich (W. Harth, Lehrstuhl f. Allg. Elektrotechnik der Technischen Universiät München, Arcisstrasse 21, D-8000 Munich 2, Germany).

14–16 September, **3rd National Conference on Quantum Physics,** Southampton (Meetings Officer, The Institute of Physics, 47 Belgrave Square, London, UK).

15 September, **Pressure Vessel Codes—Change and Advance,** London (The Institution of Mechanical Engineers, 1 Birdcage Walk, London, UK).

15–16 September, **Conference of the Schizophrenia Association of Great Britain,** London (Hon. Secretary, Tyr Twr, Llanfair Hall, Caernarvon, Wales).

Reports and Publications

UK & Ireland—June

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nature

18 August 1977

A message for them . . . or for us?

The second Voyager spacecraft is due to be launched on its trip to the outer planets on 20 August, followed 12 days later by the first Voyager spacecraft. This apparent paradox is not due to a delay of Voyager I but simply reflects the planned arrival times of the two spacecraft at Jupiter. Voyager I, having taken a faster route, should arrive four months ahead of Voyager II. The nomenclature is therefore sensible. But is the better-known decision, to place a phonograph record on board each spacecraft, also sensible?

As the two spacecraft will fly past the outer planets and then leave the solar system for ever to wander amongst the stars, this provides a unique opportunity to signal our presence to anyone out there by using the Voyager mission as an interstellar message in a bottle. The message consists of a record containing pictorial information about the solar system and its inhabitants, messages in 60 languages and a selection of music. But cold calculation shows that the probability of this message being picked up in the awesome emptiness of space is negligibly small. Measured by its chance of success, this is a futile gesture.

Nevertheless the gesture is worth making; it is an exercise designed more for the benefit of the sender than the supposed recipient and, as Carl Sagan puts it, "the launching of this bottle into the cosmic ocean says something very hopeful about life on this planet". It is an act as apparently pointless but as understandable as leaving a flag on a conquered mountain peak. Objectively the space in the climber's rucksack would be better occupied by an extra pair of socks but, such is the essential optimism and arrogance of the human race, no one objects to such acts.

We can take comfort too from the contents of our message. President Carter states on the record: "We human beings are still divided into nation states, but these states are rapidly becoming a single global civilisation". Presumably he is working on a cosmic time scale—or maybe he knows something we don't. But this process cannot have been accelerated by the omission of reference to the Soviet Union on the record. Although NASA points out that a picture of sprinters has "Valeri Borzov in the lead", this cosmic gesture has missed the opportunity to make a worthwhile worldly one.

Voyager is more than an interstellar envelope, of course, being part of a programme of planetary exploration. But the programme's future is far from certain, so the inclusion on the Voyager records of the names of the members of the US Congress may ensure a sympathetic response in certain quarters. Similarly, with the costs so great continued public interest is a vital prerequisite for the acceptability of future missions, as Viking showed; the very name Voyager was chosen to fire the public imagination, and NASA takes seriously and performs professionally its tactical duty to educate and explain.

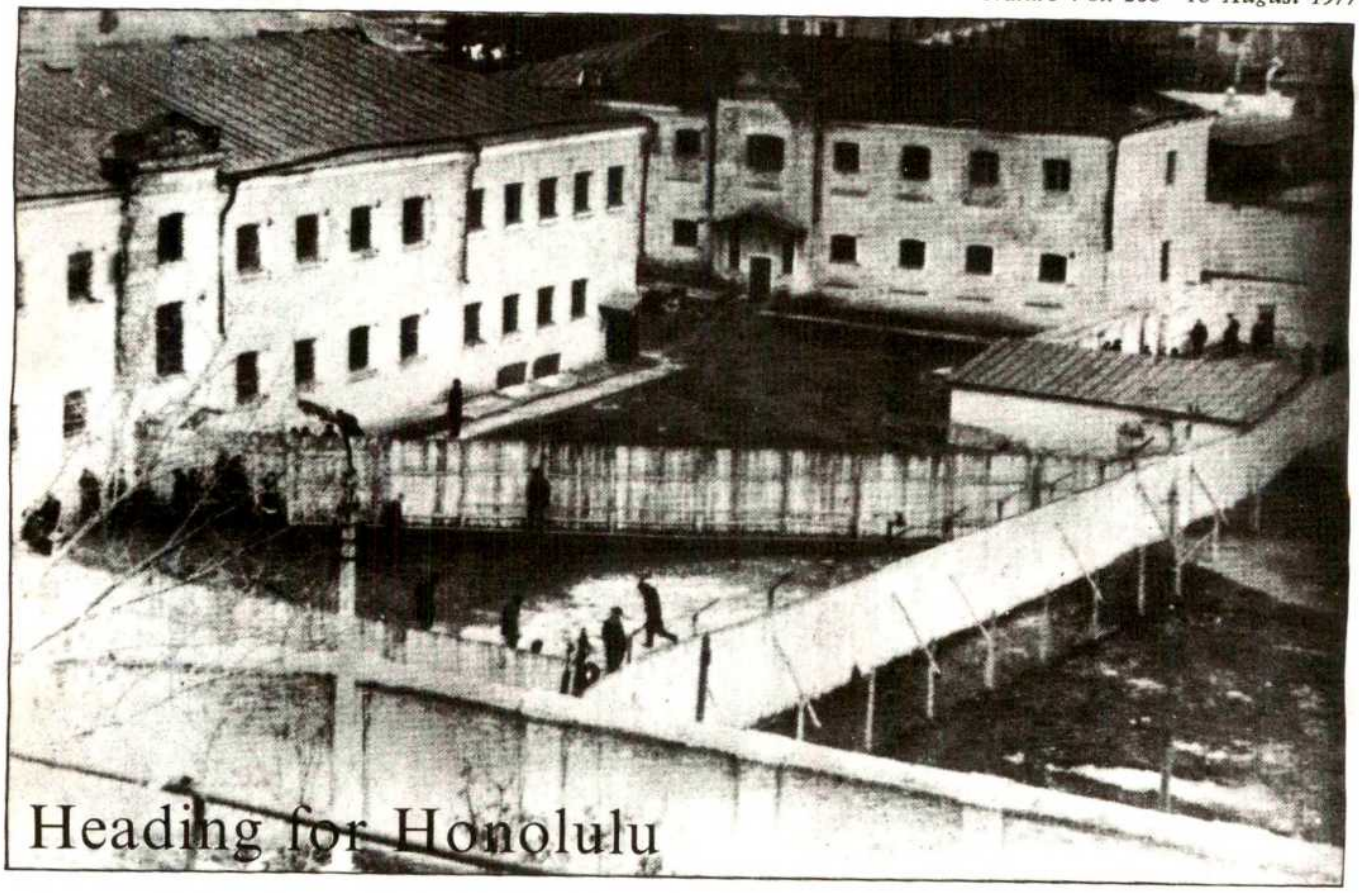
How much more than an interstellar envelope Voyager is, however, is too easily overlooked. The continued emphasis on the search for life has its dangers and has now reached the stage where it has become a betrayal of the broader aims of the scientific community. The tactic did not work with Viking, the common conclusion being that it was an expensive mission that did not provide a clear answer to the one question it supposedly set out to solve. That defines Viking as a failure. Using a similar motivation for Voyager would be a mistake.

NASA does not of course claim that the inclusion of a message is in any sense the motive for the mission, but it is giving the message wide publicity. And this is where the danger lies. The science correspondent who has the tough task of explaining a complicated topic to the public has been handed a gift on a plate: a sellable subject that is easy to write about and, more importantly, liable to get into print. Most science stories get written but not printed. Science writers can use the 'search for life' peg to get a story accepted and then produce a balanced report.

But how many will do this? It happened all too rarely with Viking outside the specialist science press, and so far it has not happened widely with Voyager. The same problem occurs outside the space programme too: how often have we been told, for example, that current research on fusion energy promises inexhaustible supplies of fuel with no radioactive waste? Such myths may help with funding in the short term, but the eventual backlash could be serious.

By the way, we understand that the Voyager spacecraft will send back some sort of data as they pass the outer planets carrying our message into the void. □

Heading for Honolulu



Orel Prison Hospital, USSR
(Copyright Peter Reddaway)

The World Psychiatric Association is about to discuss the misuse of psychiatry. **Vera Rich** considers the Soviet example

WHEN the Sixth World Congress of the World Psychiatric Association (WPA) opens in Honolulu on 28 August, one of the major issues it will discuss is the misuse of psychiatry as a means of repressing political dissent. But with opinions divided over the proper measures needed to combat this abuse, the Congress could face unprecedented controversy.

Some of the seeds of this controversy are to be found in the Soviet Union. Over the past few years, a growing body of information has reached the outside world on the confinement of political dissidents in Soviet mental hospitals. The political abuse of psychiatry is not a specifically Soviet phenomenon—there are reports of 'psychiatric torture' in certain countries of South America, and occasional reports from Eastern Europe of psychiatric measures being taken against would-be emigrants or other protesters. No other country, however, provides such a bulk of material, nor so many well-documented cases.

Public awareness in the West was first drawn to the possibility of political misuse of psychiatry in the Soviet Union in 1965, with the publication in Britain of a book by Valerii Tarsis entitled *Ward 7*. This work was essentially an autobiography disguised as a novel. Within a few years, several individual cases of such abuse made headline news—notably those of Aleksandr Esenin-Volpin, the mathematician, and Zhores Medvedev, the geneticist. The full extent of the problem only became apparent, however, in March 1971, when the International Committee for the Defence of Human Rights in Paris received over 150 pages of documentation, including what were claimed to be exact copies of official forensic reports on dissidents.

Scrutinised

During the course of the year the documents, which became known as the Bukovskii papers (after their compiler), were scrutinised by Sovietologists and psychiatrists. When the WPA held its Fifth Congress in Mexico in November 1971, strenuous efforts were made to discuss the Bukovskii material. Any firm action, however, was blocked by the Secretary-General of the WPA, Dr Denis Leigh, who averred that nothing in the WPA statutes mentioned that the WPA was responsible for ethical aspects of psychiatry. Nor, he suggested, was there any statute relating to complaints made by one member society against another.

These claims do not seem to agree with the provision in the statutes that indicates membership of the WPA, both for individuals and societies, may be terminated *inter alia* by suspension. If the WPA cannot deal with such complaints, it is difficult to see on what grounds a member society can be suspended. Dr Leigh contented himself by announcing that the complaints had been "referred to the appropriate quarter, in this case the All-Union Society of Neuropathologists and Psychiatrists"—the very body being complained about. An Ethical Committee was set up to discuss the ethics of psychiatric practice and to draw up an appropriate code of behaviour, to be discussed at the next Congress.

This decision did little to ameliorate things. Indeed, according to former internees, including Bukovskii himself and Viktor Fainberg, conditions for dissidents in mental hospitals, which had been relaxed somewhat immediately prior to the Mexico meeting, became far more stringent once it was clear that the WPA was not prepared as a body to make a stand on this issue. Dr Gary Low-Beer of Horton Hospital, an active member of the Working Group on the Internment of Dissenters in Mental Hospitals, commented that it was not necessary to have a committee to define the ethics of the issue; what was needed was some means of enforcing the accepted ethical standards.

Since the Mexico Congress, the

Bukovskii evidence has been substantiated by a number of other dissident sources, including two Soviet psychiatrists—Semeon Gluzman (now in a labour camp), who exposed the official chicanery that led to a diagnosis of insanity in the case of General Grigorenko, and Marina Voikhanskaya (now resident in London), who refused to administer punitive doses of psychotropic drugs to sane dissidents. According to Dr Leigh himself, even the Soviet delegates at Mexico were prepared to concede that the case reports in the Bukovskii material were authentic. More recently, the Soviet line when releasing a dissident has been that he or she had been insane but was now cured by Soviet medicine. Independent examination of those internees released to the West immediately after discharge from 'hospital' fail to show, however, that any psychosis had been present.

Pattern well known

The general pattern of confinement of political and religious dissidents is now well known: massive punitive doses of drugs, physical restraint, including wrapping the patient in wet canvas which shrinks as it dries, brutal treatment by convict-warders and so on. What still remains in doubt is the stand the WPA should and will take in the matter.

The report of the Ethical Committee will be presented at Honolulu and debated in plenary session. This document will cover a number of issues, including questions of consent to radical forms of treatment, safeguards in the case of involuntary commital, and so forth. So far as is known, the problem of political misuse does not feature specifically, but is contained implicitly in the topic of when treatment is not indicated.

At the General Assembly, however, which represents all 75 member societies, the problem will be specifically raised: the Royal College of Psychiatrists is presenting a motion that the WPA should condemn the Soviet practice of confining dissidents in psychiatric institutions. This motion is not likely to pass unopposed; a number of psychiatrists would prefer to keep 'political issues' out of the Congress, fearing that if the Russians walk out in protest, it would destroy any possibility of diplomatic negotiation. Commenting on this approach, Dr Harold Merskey of the University of Western Ontario observes that the WPA is not an international body like the UN, whose sole purpose is to keep diplomatic channels open; it is a professional body, he says, representing a profession in which ethical standards are all-important in any meaningful international exchange. He further remarks that "if politics have appeared in the matter, it is the Russians who have introduced them".

A special meeting to discuss individual abuses has been called on the initiative of the American Psychiatric Association; Dr Voikhanskaya is to be one of the floor speakers. The French Committee against Psychiatric Abuse is planning a rally outside the official context of the Congress. The problem underlying all such discussions and protests still remains unsolved: how far is the Soviet psychiatric profession committed to such abuses? And, following from this, what should the stance of individual psychiatrists and psychiatric bodies abroad be?

During the past few years, there has been a certain 'liberalisation' of Soviet psychological and psychiatric theory. This is reflected even in the new (1975) edition of the Large Soviet Encyclopædia. Whereas the previous edition (1955) dismisses psychoanalysis as a "bourgeois pseudo-science", the new version, though still condemning "idealistic" tendencies such as "Freudism", nevertheless acknowledges that the analytic approach has brought into scientific consideration such "important phenomena" as unconscious processes and the effect of the experiences of early childhood.

The historical survey places far less emphasis on Pavlov, who does not even merit a special biographical note, and the former description of psychiatric treatment as the eradication of harmful reflexes and the implantation of healthy ones has been replaced by a less simplistic one. Nor is it any longer stated that psychological "reality" must be understood in the sence of Marxist-Leninist reality. At first glance, the new entries seem almost as hopeful for Soviet psychiatry as the refutation of Lysenkoism was for genetics.

Marxist–Leninist science

Nevertheless, since Marxist–Leninism is considered to be a science in its own right and the basis of all Soviet science the outlook is not promising for those psychiatric 'patients' who hold opposing philosophies. The somewhat bizarre diagnoses such as "schizophrenia with delusions of reformism" may, in practice, be simply a useful tag for dropping a political nuisance into a psychiatric oubliette; but the Soviet psychiatric system provides a background that is particularly amenable to such distortions. The Soviet physicians' oath contains a pledge "to be guided by the principles of communist morality"—principles which specifically put the good of the community above that of the individual.

Certainly, many Soviet psychiatrists are unaware of the abuses. Dr Voikhanskaya states that she herself knew nothing of them for almost ten years; others, at great personal risk, refuse to participate in them. The problem of political psychiatric abuse in the Soviet Union is not, however, simply that of a relatively small number of psychiatrists putting their devotion to the establishment above their professional commitment; any serious consideration of the problem, or plan to protest, must pay serious attention to the background against which they arise and within which they can acquire some kind of 'socio-political' justification. ☐

Bukovskii: sent documents

Voikhanskaya: son still in USSR

Gluzman: still held
(This picture and picture opposite courtesy of Peter Reddaway and Sidney Bloch, authors of Russia's Political Hospitals, *published by Gollancz)*

TAIWAN

Losing representation

The council of the International Union of Geodesy and Geophysics (IUGG) met in Durham recently. David Davies reports

In a decision that seems to have been reached amid some concern about the politicisation of international non-governmental scientific organisations, the Council of the IUGG at an extraordinary meeting held in Durham has voted 47–7 to cancel the membership of Taiwan and to bring in the People's Republic of China. The vote is particularly important because it could open the way to much wider Chinese membership of international organisations, though presumably only at the price of much wider expulsion of Taiwan.

Last year the International Union of Geological Science meeting in Australia took a similar course, but the Australian government had not allowed Taiwanese representatives entry visas to state their case. In Durham Professor Y. B. Tsai spoke for Taiwan, but returned with a colleague to Taipei after the ratification of the decision, even though he was scheduled to speak at a subsequent scientific meeting.

The ballot was secret but there is no doubt that the UK and United States voted against. Both these countries stressed that they had no opposition at all to Peking's membership, but only to the preconditions. Indeed, the UK tried without success to get a motion considered which simply admitted Peking whilst taking no action on Taipei. In the event it was clear that many representatives had come with the firmest instructions from their governments or academies to admit Peking on its own terms. A few countries did not even send a scientist to vote for them but despatched an embassy official from London—a practice which the International Council of Scientific Unions would do well to look at as soon as possible.

In hindsight the haste with which the IUGG seems to have brought the matter to a head was probably unnecessary. The next IUGG assembly is planned for Australia in 1979 and it was thought at one time that Australia would refuse to support a meeting at which Taipei but not Peking was represented. But the Australian position has since softened somewhat and it is possible that the Taiwanese would have been admitted provided they did not claim to be official representatives.

The Taiwan position is difficult now, in spite of assurances that the Bureau of the IUGG will look after the interests of individuals and will ensure that appropriate information gets through. Many Durham delegates urged the Taiwanese to stay, to continue to attend meetings and to remain in the closest touch. But there is a political reason why this is unlikely. The motion that was passed did not simply call for a switch of membership —it pointedly went out of its way to explain the reason. that the "vast majority of countries" now recognise Taiwan to be a province of the People's Republic of China. The Taiwan government also claims that Taiwan is a province of China—but not, of course of the People's Republic. And the Academia Sinica of Taipei is on record as having claimed to speak only for scientists within its own shores. Thus any organisation which acknowledges *de jure* Peking rule over Taipei is unlikely to see Taiwanese citizens at its meetings in the forseeable future.

The whole sad affair, which gives the impression of having been pushed through for political convenience, will undoubtedly give people ideas about getting rid of other countries because their policies are disagreeable or their political base is weak. □

SWEDEN

Spending a packet

Wendy Barnaby reports from Stockholm on one aspect of Sweden's continuing nuclear debate

Last March, the Swedish branch of the Friends of the Earth sent a letter to the Prime Minister complaining that a so-called study packet on energy, produced with government funds, was in fact propaganda for nuclear power. The letter provoked some interest. The state auditors delved a little into the financing of the agency distributing the packet, Centrala Driftledningen (CDL), a non profit-making body which promotes many-sided cooperation between the electricity companies, and which is half financed by the State Power Board. The Prime Minister, Thorbjörn Fälldin, replied that he had not known about the distribution of the packet, but that to control the agency's operations would conflict with democratic principles. A question was asked in parliament, but received only a vague answer from the Energy Minister. Then recently—just before the government was to approve the State Power Board's annual budget—rumours circulated that its normal contribution of about $865,000 to CDL would be axed. In the event, the money was approved and the agency will be able to continue its normal operations.

The study packet was written by CDL and various organisations responsible for energy supplies. Meant for schools, study circles, trade unions, companies and so on, it consists of seven attractive booklets totalling 152 pages, as well as a 102-page book entitled *Nuclear Energy: Principles and Problems* written by a reactor physicist who works with the Atomic Energy Company. Describing the aims of the package, the distributors said that its facts should be correct and easily distinguishable from evaluations.

In some cases, this holds; often it does not. One of the booklets stresses, for example, that no increase in genetic defects has been found in the offspring of survivors of Hiroshima and Nagasaki. But for a public generally ignorant of the studies showing that the survivors have more than their share of medical complaints, the suspicion arises that the authors are trying to minimise the effects of radiation. The same booklet also asserts that the risks of nuclear reactors are small, that the industry is subjected to stricter control than any other, and that the dangers of nuclear-weapon proliferation and terrorism have been exaggerated. In short, the publications, taken together, leave no doubt that the best available energy source is nuclear.

Following the news that CDL would be able to continue, its director, Bengt Sterne, commented: "It was a political question—it's all over now". Of course it *was* a political question. The President of Friends of the Earth, Lennart Daléus, agrees in principle that CDL should have its money, but says that, as it is the only organisation with enough money to give the public significant amounts of information, it should use government funds more responsibly. Certainly, the problem would be eased if both sides of the nuclear debate could compete on equal terms. It has long been a sore point with antinuclear groups that government support is so one-sided. A spokesman at the Department of Industry, which also deals with energy, says that at the moment there is no government funding of anti-nuclear groups—although there is about $100,000 in the pipeline for them. A proposal by the Liberal Party that they should get an annual grant of about $22,000 seems to have been dropped. Even if it had been adopted, total government spending would still have favoured the pro-nuclear groups by about eleven to one. □

NETHERLANDS

●Mrs Vorrink, Holland's Minister of Public Health and Environmental Protection, plans to eliminate the presence of phosphates in detergents by 1985, according to a report just published by the ministry and already agreed upon in a coordination-committee in which all departments take part. Partial or regional bans operate in Canada and the US; Germany plans to cut back the phosphate content in detergents to half the present level.

The problem is international, and in each country is caused partly by pollution from abroad. In the Netherlands more than half of the phosphates in the surface-waters is imported from other countries, and of the rest a good portion comes from detergents. The initiative to ban phosphates will help, but a more complete solution would require European action. Industry's assistance would also be necessary.

Before Mrs Vorrink reached the end of her ministerial appointment—she is unlikely to return in the new government now in the process of formation—she concerned herself with the warning on spray cans which contain halocarbons. The warning mentions that they can have deleterious environmental effects. The minister's memorandum announces a ban on the use of the gases in spray cans. Industry protested immediately, saying that work for 700 people would be in danger and that the measures would be premature, as no scientific evidence was available that the ozone layer is broken down by halocarbons.

●The former Minister for Science Policy, Mr Trip, will not be part of a new government. What is more, his portfolio is said to be in danger, in spite of advice to the Prime Minister, Mr den Uyl. He has received two reports on improving the communication of information on science and technology to a larger public. One was compiled by two committees, in which scientists, science-information officers and science writers participated.

The product of 18 months' work, the report concluded that a central service for information on science should be created at the Royal Academy of Sciences, which should have a coordinating role. In the Netherlands all universities have their press- and information-offices; so too do most of the government research institutes. The position has improved in recent years, but gaps remain. Information is restricted mainly to the institutes' research, and no connection exists with research work elsewhere. The new service has to work as independently as possible and aims at objectivity.

With so many groups working in this area in the Netherlands already, Mr Trip formed another committee to examine whether the government should subsidise existing activities, particularly of foundations which promoted information based on a particular view on society. The committee considered that critical evaluations of the development of science and technology were necessary, and the minister says in his recommendations that more participatory forms of discussion on science are needed, involving various organisations and groups in society. The committee recommends that government money be used for this, without offering criteria for its disbursement. Thus a 'Science for Citizens' programme which is already carried out on a modest scale in the Netherlands may now grow larger.

●Alternative forms of energy receive much attention in this most densely populated country in the world. After all, nuclear power is not very popular: the construction of three 1,000 MW power plants, and the extension of the ultracentrifuge uranium enrichment plant at Almelo which is part of the tripartite consortium with Britain and Germany, form one of the points of discussion about the formation of a new government. Nuclear matters still dominate research, but alternative energies may obtain a foothold. The minister who has energy in his portfolio, Mr Lubbers of Economic Affairs, is a proponent of nuclear energy on what he calls a modest scale. He has put into service an experimental wind turbine, part of a five-year 15-million-guilder national research programme on wind energy.

The experimental wind turbine, 5 m in diameter, is of the Darreus-type and was built by Fokker-VFW as a spin-off of the rotorblade-production for helicopters. If it is successful a wind turbine 25 m in diameter will be built as well as a more classic wind-turbine of the same size. Mr Lubbers sees only a restricted role for wind energy, however, especially with the environmental and country-planning problems. Experts expect more than 10–20% of future electricity production to come from the wind.

The problem seems even greater with solar energy, but in fact some 50 houses with solar collectors and other energy saving devices have been built in Holland during the last three years. An ecological group, 'De Kleine Aarde' (the small-scale world), began with a recycling house five years ago and the reseach of a professor at Eindhoven Technical University who lives with his family in a solar house was a stimulus. Philips is doing experiments on 32 houses with collectors; an office building will be constucted with collectors; and this month four houses have been finished in which technical collaborators of the national research organisation will live with their instruments.

The research centre for the building industry foresees the introduction of solar energy devices in houses for space heating and production of warm water from 1980. A national symposium will be held next month on the development of solar energy in the Netherlands.

Casper Schuuring

IN BRIEF

SA nuclear alarm

The question mark surrounding the development of South Africa's capacity to produce nuclear weapons was brought more fully into relief last week following allegations made by the Soviet news agency Tass that South Africa was preparing to carry out weapons tests. The allegations anticipated the rush of diplomatic moves over Southern Africa late last week, and Dr Owen, the UK Foreign Secretary, and Herr Genscher, West Germany's Foreign Minister, each brought the subject up with their South African counterpart, Mr Pik Botha.

Bonn this week denied more reports of collaboration with South Africa on nuclear development. Like allegations earlier this year relating to contacts on nuclear matters between the two countries, these were based on documents reportedly in the possession of the African National Congress, the exiled black nationalist organisation based in Lusaka. According to the *Observer*, the ANC says that many military visits to Germany have been made under the cover of the South African Council for Scientific and Industrial Research, and are arranged through the scientific counsellor at the Bonn Embassy. ANC officials are also reported as saying that 15 German research institutions, including the Max Planck Institute for Nuclear Physics at Heidelberg, have contributed to South Africa's arms programme.

Committee's report

In its latest report, the UK Select Committee on Science and Technology has examined a particular instance in which British industry did not exploit a potentially useful innovation. Called 'A case study of machine tool development at the Cranfield Institute of Technology', the report outlines how a high-precision electronic formed-wheel gear-grinding machine, developed at Cranfield Unit for Precision Engineering and Matrix Machine Tools Ltd, failed to gain the financial support needed to develop it commercially.

Blame for this failure is distributed among most of those concerned with the project, though the committee admits that no one acted totally unreasonably. It criticises the Department of Industry's Mechanical Engineering and Machine Tools Requirements Board for not seeking more evidence on the machine's potential market; and the industries involved are criticised for not considering developing the machine as a joint venture.

Berkeley women

THOMAS H. JUKES

THE University of California at Berkeley has long prided itself as being a citadel where liberal causes and freedom of thought, action and expression were fostered. Fostered, that is, by a predominantly male faculty. Women did the typing. A less-publicised aspect of campus life at Berkeley is that many women students feel personally insecure. These circumstances lend unusual interest to the appearance of a report, *Women Students at Berkeley: Views and Data on Possible Sex Discrimination in Academic Programs,* that has just been issued by the Office of the Chancellor; 140 pages long, it contains excerpts from academic units' self-evaluation reports and from students' responses to questionnaires.

Chairmen of departments, and other male administrators, made responses that were in many cases masterpieces of low-key diplomacy. The devotion of some respondents to women's liberation was exceeded only by the complete, or nearly complete, absence of women from regular faculty appointments in their departments. Most of us are familiar with the pre-emption of certain scientific fields, such as engineering, by males. It is also evident that physics, chemistry, astronomy and mathematics are predominantly male worlds, in which barriers against women are apparently raised long before college age. However, it surprised me to learn that "Palaeontology is simply a field of endeavour that fewer women than men find attractive, and it is naive to believe that the current disparity in numbers of women to men in the field can be brought to parity by governmental fiat".

Space permits only a few highlights. The Chairman of the Department of Dramatic Arts notes that "Drama, for 2,000 years, has been predominantly written and acted by males . . . Hamlet is a better role than Gertrude, and there are over 30 men and two women in the cast". It might be retorted that Portia, in *The Merchant of Venice,* was much better at making up her mind and getting results than was the moody and vacillating Dane. Also, transvestite males filled women's roles on the stage of Shakespeare's day. Why should not the reverse procedure be tried now?

A laboratory director says, "In all fairness, it must be pointed out that the natural environmental conditions at the High Altitude Laboratories are physiologically stressful, and for whatever the underlying sociological reasons, the use of the facilities by men has been substantially greater than by women". We hope that his laboratory will provide experimental evidence for the interesting implication that females are more affected by anoxia than are males. I believe that experimental subjects could readily be obtained, for US Governmental Affirmative Action policies have brought many female rangers to the National Parks and Forest Service areas close to the High Altitude Research Laboratory. I have encountered these athletic young females striding along the trails at elevations above 11,000 feet, apparently without signs of stress. Also, women members of the American Alpine Club are more tolerant of high altitudes than they are of suggestions that they are less able than males to adapt to them.

Medicine is a famous battleground of the sexes, and the early women MDs are revered as much as freedom fighters as for their clinical prowess. As every medical student knows, many professors in the clinical sciences are masterful male chauvinists. The report contains several citations of this, and also the following gem: "A neurologist described the Argyll-Robertson pupil 'like a whore who accommodates but does not react' ".

Forty-six of the fifty-six female students responding to the questionnaire expressed fear of being raped on the campus at night. Practically none of their professors even mentioned this problem.

news and views

Optimal foraging: theory and experiment

from John Krebs

It may seem a bit optimistic to try and predict in theory how an optimally efficient predator ought to behave, and then expect the predictions of the model to work. Nevertheless a growing body of experiment indicates that the decision rules used by predators are often surprisingly close to those predicted by simple models.

The idea of trying to formulate decision rules for optimally efficient predators was suggested 11 years ago by R. H. MacArthur and E. R. Pianka (*Am. Nat.* **100** 603; 1966) who proposed that predators (using this term in a very broad sense) are designed by natural selection to maximise their net rate of food intake while foraging. They discussed in these terms how an ideal predator might make choices about which food items to eat and where to forage. E. L. Charnov, J. M. Emlen and T. W. Schoener are among several theoreticians who have followed up MacArthur's and Pianka's ideas, and experimental work designed to test the theory has been done, mainly in the laboratory, with birds, arthropods and fish.

Imagine a bird faced with a variety of seeds of different sizes. Ignoring for the moment considerations such as taste and presence or absence of trace nutrients, an optimally efficient bird ought to be capable of ranking the seed types in terms of profitability – the net quantity of food extracted per second of handling time – and when given a straight choice it should prefer the most profitable seeds. There are several studies which have confirmed this basic assumption of optimal diet models for a variety of predators. The predictions of the models relate the extent to which the predator should select only profitable prey and the rate of encounter with the various prey types. In general, predators should be more selective when profitable prey are encountered frequently than when they are rare. (Note that the term 'encounter rate' covers the effects of camouflage and so on.) This makes intuitive sense. When the predator pauses to handle an unprofitable item, it uses up time which could otherwise be spent in travelling on to the next prey. If profitable prey are sufficiently common it will always be economically advantageous to pass over the less profitable prey. On the other hand in a time of shortage even unprofitable prey may boost the predator's average rate of intake. More precisely, the rule for optimal choice of food items is that the predator should add a new prey type to its diet only if the net food yield per second of handling time (E/h) for this prey type exceeds the predator's current overall net rate of food intake. This prediction can be tested quantitatively by measuring the food values (usually considered in terms of weight or energy), handling times, and encounter rates with various prey types. Particularly noteworthy is the prediction that a predator's decision to accept or reject a new prey type ought to depend only on the value of E/h for the new prey and not on the encounter rate. In other words, there should be (exactly specifiable) conditions in which a predator ignores very common, unprofitable prey.

The importance of this last point can be seen by referring to an experimental test of optimal diet theory in which Werner and Hall (*Ecology* **55**, 1042; 1974) examined the selection of different sizes of *Daphnia* by bluegill sunfish (*Lepomis macrochirus*). Werner and Hall allowed small groups of sunfish to forage in a large tank containing a known mixture of *Daphnia* of varying sizes. They estimated the encounter rate with each size class by making some simple assumptions about the visual field of the fish, and were then able to predict the optimal contents of the sunfishes' diet on the basis of the relative profitability of different sized prey. Large *Daphnia* are more profitable than smaller ones because they contain more food but take no longer to handle. Werner and Hall found that when all size classes were available at a low density the fish were totally non-selective, but when the density of all size classes was increased, the fish selectively ignored small *Daphnia*. This switch in behaviour occurred at the encounter rate with large *Daphnia* predicted by the optimal diet model.

More recently O'Brien *et al.* (*Ecology* **57**, 1304; 1976) have pointed out that Werner's and Hall's results could equally well be explained by assuming that the fish obey the rule of always taking the *Daphnia* with the largest apparent size (a small *Daphnia* close by might appear larger than a big one at a distance). O'Brien *et al.* showed that sunfish do in fact take the apparently larger of the two *Daphnia* in a simultaneous choice experiment, and they incorporated these results into a simulation model which predicted a change from non-selective predation at the encounter rate observed by Werner and Hall.

O'Brien *et al.* view their results as demonstrating a proximate rule by which the sunfish achieve the ultimate goal of optimal foraging, but this may be incorrect. The 'take the largest' rule, in agreement with the optimal diet model, predicts that the fish should become more selective as the density of large *Daphnia* is increased, but the two models differ in their prediction of what ought to happen if now the density of small prey is increased enormously. The optimal diet model says that the fish ought to continue to ignore small *Daphnia* even when they are much commoner than large prey, as long as the density of large prey is above the selection threshold. In contrast, the 'take the largest' rule predicts that as the small prey become more abundant, they are more likely to appear larger than big prey to the fish, and hence be selected.

Werner and Hall did not do the test which would enable one to decide

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whether sunfish use the 'take the largest' rule, or the optimal diet rule. Two more recent studies have, however, shown that two species of bird will ignore very abundant but unprofitable prey when the encounter rate with profitable sizes is above the predicted level. Goss Custard (*Anim. Behav.* **25**, 10; 1977) showed this with redshank hunting for polychaete worms and Krebs *et al.* (*Anim. Behav.* **15**, 30; 1977) obtained similar results with captive great tits feeding on pieces of mealworm cut into different sizes.

Charnov (*Theor. Pop. Biol.* **9**, 129; 1976) J. M. Emlen (*Ecology; an evolutionary approach* Addison-Wesley, Reading, Massachusetts, 1973), Cook and Hubbard (*J. Anim. Ecol.* **46**, 115; 1977), and Parker and Stuart (*Am. Nat.* **110**, 1055; 1976) are prominent among those who have developed models for optimal allocation of time by a predator to different patches of prey. The models assume that when a predator arrives in a new patch or clump of prey (which might, for example, be a bush laden with berries) its food intake is at first high, but soon declines as the food patch is depleted. The optimal predator should leave a patch when its net rate of food intake within the patch drops to a level equal to the expected intake from the rest of the area. This latter quantity depends on both the average quality of patches and the time and energy cost of travelling between patches. (It is a weakness of both this and the optimal diet models that nothing is specified about how the predator acquires information about its average rate of intake.)

The most straightforward predictions of the model are as follows. First, if patch quality is held constant, the time spent foraging in each patch should depend on the cost of travelling between patches. The exact relationship between inter-patch travel time and time spent foraging in each patch can be predicted by measuring the curve of food intake as a function of foraging time in a patch. This has been done by Cowie (*Nature* **268**, 137; 1977) using captive great tits foraging for mealworms in sawdust-filled pots. Cowie's results show not only that the time spent in a patch is related to travel time in the way predicted by the model, but that data give a closer fit to the model if one considers net energy intake (subtracting the energy cost of travelling) rather than gross intake. The second testable prediction is that if travel time is held constant and patch quality is varied, the predator should leave each patch within an area at the same threshold rate of food intake, regardless of the initial quality of the patch. This prediction has also been tested and confirmed in laboratory experiments with birds (Krebs *et al.* *Anim. Behav.* **22**, 953; 1974).

In conclusion, although optimal foraging models are in many ways grossly oversimplified, they are good descriptions of the decision rules used by predators in simple laboratory and field conditions. There are many ways in which the models could be elaborated and made more realistic, for example by dealing with the problem of how a predator samples a changing environment in order to assess its average rate of food intake, and how the qualitative differences in food types influence its choice of an optimal diet. □

Cosmic ray composition at high energies

rom a Correspondent

THE mass composition of primary cosmic ray particles above an energy of $\sim 10^{12}$ eV still remains something of a mystery. At around 10^{11} eV per nucleon very detailed isotopic measurements have now been made, but from 10^{12} eV right up to the highest detected energies of $\sim 10^{20}$ eV per particle controversial debate still rages as to even the simplest question of the proportion of protons in the primary flux. This uncertainty does not reflect any lack of interest or experimental effort attached to the problem as the mass composition has very direct bearing on the origin of cosmic rays, a still very open question. The low primary flux and the Earth's atmosphere combine to make experimental investigation very difficult.

It has long been recognised that at $\sim 10^{11}$ eV $\sim 90\%$ of the primary flux is protons, $\sim 10\%$ α particles and $\sim 1\%$ the rest, largely reflecting the accepted Universal abundance. At $\sim 10^{12}$ eV early satellite experiments by Grigorov *et al.* (*Proc. 12th Int. Conf. on Cosmic Rays* **5**, 1746; 1971) using ionisation calorimeters suggested that the proportion of protons dropped with increasing energy. However the general assumption has usually been that the composition at least up to $\sim 10^{14}$ eV is not too different from that measured at lower energies.

A recent paper by Adair *et al.* (*Phys. Rev. Lett.* **39**, 112; 1977) seems however to support Grigorov's suggestion. This new work is concerned with information deduced from the charge ratio of secondary cosmic ray muons measured at sea level. Muons reach sea level as the decay products of π mesons and K mesons produced high in the atmosphere from interactions of cosmic ray hadrons with air nuclei. As the primary cosmic rays start off carrying a net positive charge, this is reflected in the excess of positively charged muons at sea level.

Experimentally the cosmic ray sea level muon charge ratio (μ^+/μ^-) is a slow function of sea level muon energy, rising from ~ 1.2 at 10^9 eV to ~ 1.3 at 10^{12} eV. In recent years a number of theoretical attempts have been made to explain this ratio in terms of the known composition of the primary flux and the details of the hadron-hadron interactions. Earlier this year a group from NASA, Badwher *et al.* (*Phys. Rev.* **D15**, 820; 1977), calculated the sea level differential muon momentum spectrum and charge ratio from 1 GeV/c to 5,000 GeV/c, using available accelerator data. They found excellent agreement between their calculations and existing experimental data and had no need to invoke any change either in the cosmic ray chemical composition or in the nature of the hadron–nucleus interaction at hadron energies above 1.5×10^{12} eV. This result was clearly in contradiction to some earlier conclusions of Adair (*Phys. Rev. Lett.* **33**, 115; 1974).

In view of this discrepancy Adair and his collleagues have now taken the research one stage further by investigating the problem experimentally in the laboratory by observing the charge ratio of muons produced by the interaction of the primary cosmic ray nucleons with the thick atmosphere. The experiment was conducted at Fermilab using the interaction of 400 GeV protons with thick copper targets.

In general, measurements of the charge ratio of muons produced at different energies were made by determining the intensities of positive and negative muons produced by the interaction of 400 GeV protons with copper targets of different mean densities. The charge of the muons was determined by magnetic analysis and the muon energies were measured by determining their range through steel and earth absorbers. For a very dense target the intensity of muons from meson decay is greatly suppressed relative to the atmosphere as a target and sources of muons which do not scale with density cannot be neglected. Such contamination was corrected for by use of targets of differing mean densities. The experiments produced a measured charge ratio for muons of 1.66 ± 0.09.

These measurements enabled Adair *et al.* to deduce from the cosmic ray energy spectrum that a muon charge ratio of 1.50 ± 0.08 should be obtained at sea level for cosmic ray muons of energy $>10^{11}$ eV assuming the accepted primary mass composition. This charge ratio value is significantly higher than the actual measured value at sea level of 1.30. This actual value indicates therefore that ~25% of the total primary nucleon flux must be made up of neutrons. (The primary nucleon flux includes all nucleons whether in multi-nucleon nuclei or not). In other words the flux of heavy nuclei in primary cosmic rays at energies $\gtrsim 10^{12}$ eV per nucleon must be larger than that observed at lower energies.

So this conclusion supports the earlier suggestion from the satellite work of Grigorov *et al*. Such a conclusion if confirmed will be of the utmost importance to cosmic ray physicists. ☐

Ribsomal switches

from E. G. Richards

THE notion that RNA molecules can exist in several metastable conformational states and that such conformers can be separated by electrophoresis on polyacrylamide gels is by now well known. It all began with tRNA; 5S RNA was the next to exhibit this property and more recently, 16S RNA has been observed to do the same. So far the transformation from one conformer to another has required heat or reagents such as magnesium ions or EDTA. The idea that proteins could do this has remained speculative though proteins have been described that destabilise double helical regions in RNA structures.

These speculations have recently been turned into demonstrations in a paper by Hochkeppel and Craven (*J. molec. Biol.* **113,** 623; 1977). It had previously been shown that 16S RNA isolated from the 30S ribosomal subunits of *Escherichia coli* by treatment with phenol (16S RNA^{p}) was not the same as the RNA isolated by treatment with acetic acid and urea (16S RNA^{a}): the two forms can be separated by electrophoresis in agarose/polyacrylamide and 16S RNA^{a} bound ribosomal proteins that 16S RNA^{p} did not. In their latest paper they show that if they bind several of the ribosomal proteins to 16S RNA^{p}, and then remove them again, there results RNA migrating with a mobility close to that of the slower 16S RNA^{a} rather than that of the faster moving 16S RNA^{p}. Appropriate controls established that it was not the re-extraction procedure itself that wrought this change. Proteins found to have this effect were S4, S7 and S8, but this of course does not preclude that other untried ones do the same.

These authors went on to demonstrate in a similar way that 16S RNA^{p} pretreated with S7 (the protein being bound to the RNA and then removed) was able to bind S9 whereas untreated 16S RNA^{p} was not. 16S RNA^{p} pretreated with both S4 and S7 was even more efficient at binding S9 but pretreatment with S4 alone did not affect the capacity to bind S9 even though it resulted in a lowered mobility. It would therefore seem that the different ribosomal proteins are able to bring about several different conformational states, with differential protein binding ability but all with reduced mobility.

Further experiments demonstrated that the RNA extracted from the reconstitution intermediate (RI) migrated slowly like 16S RNA^{a} while that extracted from the heat activated particle (RI*) migrated with the faster mobility. It was similarly shown that inactivation by ion depletion of intact 30S ribosomal subunits results in the extraction of RNA with a lowered mobility as well as a loss of capacity to bind tRNA in the intact particle.

Present understanding suggests that the difference between one RNA conformer and another must reside either in the pattern of base-paired helical regions constituting the secondary structure or in the pattern of folding and interaction deployed in the tertiary structure. It thus seems likely that to change one conformer into another, base pairs or tertiary interactions must first be undone. Several proteins are able to bring this about (including the ribosomal protein S1) so it is not too surprising that the ribosomal proteins, S4, S7 and S8 can do likewise. Nevertheless, the implications for the study of ribosomes are very interesting indeed.

As Hochkeppel and Craven themselves point out, it is now possible to begin to give a rational interpretation in terms of conformational changes in the RNA of the assembly maps presented by Mizushima and Nomura. One can envisage a series of conformational switches each turned on by the binding of a specific protein and each enabling the next protein to bind. If this speculation turns out to be valid, the assembly of a ribosome must be seen as a carefully orchestrated process rather than a series of random associations occurring in no fixed sequence.

The possible importance of conformational switches was discussed a few months ago by Weidner *et al.* (*Nature* **266,** 193; 1977) in a discussion of the possible conformational states of 5S RNA, another ribosomal component. Recently Bear *et al.* (*Nucleic Acids Res.* **4,** 2511; 1977) have presented some evidence that the binding of proteins L18 and L25 to 5S RNA wreaks a change in the RNA conformation. The evidence is based on changes on the circular dichroism spectra and melting curves of the RNA and RNA–protein complexes and is not wholely conclusive. It does, however, nudge one's prejudices a little further along the direction of supposing that conformational switches may be an important and widely used trick employed in a variety of dynamic molecular processes in living cells. ☐

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New sulphide mineral discovered

from Peter J. Smith

A PREVIOUSLY unknown potassium-rich sulphide mineral has been found by Clarke *et al.* (*Earth planet Sci. Lett.*, **35,** 421; 1977) within a clinopyroxene–ilmenite intergrowth from kimberlite diatremes in South Africa. Until the new sulphide has been more thoroughly investigated, Clarke and his colleagues in Canada and Scotland refrain from giving it a name, referring to it for the time being simply as FSKS (Frank Smith K-sulphide) after the Cape Province diamond mine of which the diatremes form the base. The discovery of a new mineral is not, of course, a completely unheard of event even at this late stage in the development of geology; but what makes FSKS of more than passing interest is that it may indicate an important source of potassium in the upper mantle and throw some light on the vexed question of the transfer of potassium and sulphur from the mantle to the core.

FSKS occurs as ovoid blebs (which also contain pyrrhotite and pentlandite), both as individual grains in cracks in the host clinopyroxene and, more commonly, in close association with the ilmenite lamellae. Because of its high potassium content (8.12–13.01 weight %) it inevitably invites comparison with other potassium-bearing sulphides, namely, the djerfisherite found in several meteorites, the more copper-rich djerfisherite found in the Talnakh copper deposit in Russia, and the rasvumite found in the Russian Khobina massif. But chemical analysis shows FSKS to be quite different from

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these minerals. Whereas in djerfisherite the copper contents range from 4–16% and nickel values do not exceed 4%, FSKS contains only 1–3% copper but 11–15% nickel. Moreover, it plots quite distinctly on K–Ni–Fe and K–Cu–Ni ternary diagrams not only from djerfisherite but also from rasvumite.

There seems little doubt, therefore, that FSKS is chemically distinct from other known potassium-rich sulphides. With its primitive cubic unit cell (dimension 10.29 ± 0.03 Å it is also structurally different from rasvumite, which is orthorhombic (although djerfisherite is cubic in structure). What is less certain, however, is whether or not the significance of FSKS extends beyond the particular circumstances represented by the diatremes within which the mineral is found.

Clarke and his coworkers tentatively propose three possible origins for the clinopyroxene – ilmenite – pyrrhotite – pentlandite–FSKS assemblage, whilst admitting that none is consistent with all the observations. According to the first, the clinopyroxene–ilmenite intergrowth would have grown directly from a silicate melt in the mantle and the polysulphide blebs would have been trapped as immiscible liquid droplets. The unmixing of the blebs into the three distinct phases would then probably have taken place below 600 °C, after the material had been transported to the crust in a kimberlitic fluid. And according to the second, all phases, including the ilmenite, would have been unmixed from an initially homogeneous clinopyroxene by a process of multiple exsolution, although again the separation into the three sulphide phases would have had to have taken place after emplacement in the crust. In both of these models it is implicit that the potassium was present in the initial phases. The existence of FSKS in cracks in the host clinopyroxene, however, suggests the addition of potassium at a late stage, presumably within the diatremes themselves. It is thus possible to envisage a third mechanism in which potassium-bearing sulphides are formed metasomatically at low pressure at the expense of earlier sulphide phases.

If the production of FSKS is really dependent upon diatremes, or on the Frank Smith diatremes in particular, the new mineral clearly has little relevance to the general problem of potassium in the mantle and core. This may indeed be the case, for FSKS has a chemical inhomogeneity great enough to be regarded as uncharacteristic of a phase which has been equilibrated in the mantle for long periods, suggesting that it may be a metastable material associated with processes occurring during the rise of kimberlitic fluid to the surface. On the other hand, if, as in the first two models proposed by Clarke *et al.*, FSKS is a primary material in the mantle or results from a closed-system unmixing of a monosulphide solid solution, sulphides take on a greater importance as possible storers of potassium in the upper mantle and as possible carriers of potassium to the core during the Earth's differentiation.

This raises deep issues on which it would perhaps be unwise to speculate too much until the properties of FSKS have been examined in greater detail. There has recently been considerable debate and disagreement over the role of sulphur, sulphides and potassium carriers in the Earth's lower mantle and core and any new lead in these matters would be welcome. In the meantime, we have at the very least a new natural sulphide, whatever may be its geochemical significance. ☐

Pion production in heavy-ion collisions

from P. E. Hodgson

A RECENT measurement of the frequency of pion emission from the collision of heavy ions has given results that disagree with the predictions of the independent particle model of the nucleus and support the pion condensation model of high-density nuclear states.

The frequency of pion production in free nucleon–nucleon collisions is well known, so if nuclei behave just like groups of independent nucleons it is easy to calculate the expected rate of pion production in collisions between two nuclei at high energies. This model might be expected to be valid at energies high enough for the wavelengths of the interacting nucleons to be rather less than the mean separation of the nucleons in the nucleus. If this is so, the nuclei behave for most purposes as if they are groups of independent nucleons, and the expected rate of pion production is given by the total number of possible nucleon–nucleon interactions, making allowance for multiple scattering and shadowing effects. Significant deviations from the expected number of pions would indicate the presence of strong interactions between the nucleons in the interacting nuclei.

The experimental determination of the pion production rate was made by McNulty *et al.* (*Phys. Rev. Lett.* **38**, 1519, 1977). They exposed a stack of G5 nuclear emulsion to a beam of 280 MeV per nucleon neon nuclei at the Princeton accelerator. Examination of the developed emulsion under the microscope showed the tracks of these neon nuclei, and 189 tracks were found of nuclei that had collided with a nucleus in the emulsion. Many pion tracks were observed originating in these collision events, and careful subsidiary measurements established that pion tracks could be distinguished from those of protons and alpha particles. The results for the rate of pion production as a function of the energy of the neon nuclei, and also for the pion energy spectrum, are shown in the figures.

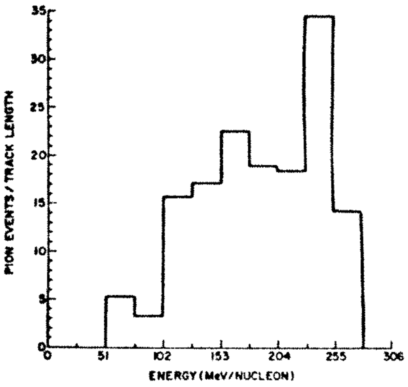

Fig. 1 Pion production as function of neon energy.

A calculation of the rate of pion production expected from the independent particle model has recently been made by Bertsch. He found that the fraction of collisions from which pions emerge should increase from about 1 in 3,000 at the threshold energy of about 54 MeV to about 1 in 27 at energies of 250 MeV per nucleon. This is in complete disagreement with the results shown in the first figure, which show that about 70% of collisions at energies between 100 and 280 MeV per nucleon show pion emission.

Some other calculations of pion production in heavy-ion collisions have been made by Kitazoe *et al.* (*Lett. Nuovo Cim.* **13**, 139; 1975; *Lett. Nuovo Cim.* **14**, 400, 407; 1975) using the

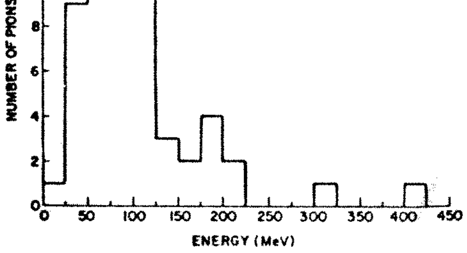

Fig. 2 Pion energy spectrum.

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hydrodynamic model to include the collective effects on the formation of high-density states in nuclear matter in high energy heavy-ion collisions. In such collisions it is possible for nuclear matter to be compressed momentarily to very high densities, and this could have a considerable effect on the rate of pion production. The high densities are associated with high temperatures, and these can result in pion condensation in just the same way as a gas is ionised with the liberation of electrons when its temperature and pressure are raised. The probability of pion formation can be calculated from statistical theory assuming thermodynamic equilibrium between the pion and nucleon components. In a later paper they reformulated the theory relativistically and evaluated the rate of pion production quantum mechanically.

In particular they calculated the effect of the condensation of zero-momentum pions, and found that this might be appreciable at incident energies of 120 to 440 MeV per nucleon, reaching a peak at an incident energy of about 220 MeV per nucleon. They estimate that as many pions as 0.4 per nucleon may be produced and allowing for those that do not escape from the nucleus gives a result in agreement with the experimental results.

These calculations are still in an early stage, but it is notable that they give results similar to those found experimentally, while those based on the independent particle model are far too low. This indicates that some new process is taking place in energetic collisions between heavy ions, and this should be a strong stimulus to further experimental and theoretical work. ☐

The oddity of blue

from J. D. Mollon

A working party was held in Cambridge in 12-14 July, 1977 to discuss the anomalies presented by the 'blue mechanism' of man's colour vision. The meeting was held at the Kenneth Craik Laboratory, an interdisciplinary laboratory for sensory science, recently founded by the Departments of Physiology and Experimental Psychology, and was supported by a grant from the Cambridge Philosophical Society.

THE first stage of man's colour vision is relatively well understood. It is generally held that there are just three classes of retinal cone, each sensitive to a broad band of the spectrum; and that all our varied experience of hue depends on the comparison, by later neural mechanisms, of the outputs of these receptor cells, each of which is individually colour blind. However, it is increasingly difficult to neglect a catalogue of signs that the blue-sensitive receptor system differs from the green and red-sensitive systems. By special sensory experiments it is possible to study our visual capacities when stimuli are detected only by blue cones and the 'blue mechanism' defined by these experiments turns out to have many odd properties. Moreover, the blue mechanism is disproportionately vulnerable to retinal disease. The working party was held to attempt to sort out these anomalies.

From the discussions emerged three explanatory principles that may account for most of the anomalies of the blue mechanism. Firstly, there are probably rather few blue-sensitive cones. This might explain the poor quantum efficiency and the poor spatial resolution that characterise the blue mechanism as well as the fact that normal observers when matching very small or brief targets resemble the rare class of congenitally colour-blind observers ('tritanopes') who seem to lack blue cones. H. G. Sperling (University of Texas) presented evidence for the relative rarity of blue cones in the baboon retina: measuring the light-induced reduction of nitroblue tetrazolium chloride in the ellipsoids of cones, he found that the putative blue cones (those that stain strongly when exposed to blue light) constitute 12% of the total population of cones over much of the retina and are arranged in a regular mosaic. D. MacLeod, M. Hayhoe and D. Williams (University of California) have demonstrated sharp local variations in the threshold sensitivity of the blue mechanism within an individual human fovea. They also confirmed the finding of Willmer that the very centre of the fovea essentially lacks the blue mechanism. Correspondingly Sperling's measurements suggest that the proportion of blue cones falls to 3–4% at the centre of the fovea.

A second explanatory hypothesis is that signals from blue cones have access only to the 'chromatically opponent' pathways of the visual system, which are thought to carry information about colour. P. Gouras and E. Zrenner (National Institutes of Health, Bethesda) have detected blue-cone inputs in only one class of retinal ganglion cell, which are characterised by tonic responses to blue light and off-responses to long wavelengths. P. Whittle (University of Cambridge) tackled the related and vexed, question of whether the blue cones contribute to brightness as well as to hue: presenting to one eye a flash detectable only by blue clones and to the other a white flash on a white field, he found that some subjects were able to equate the two flashes in brightness while others found the task meaningless.

If indeed signals from blue cones are transmitted only by chromatically opponent pathways, it might be expected that the sensitivity of the psychophysically-defined blue mechanism would be controlled not only by photons absorbed directly in the blue cones but also by signals from long-wavelength cones. E. N. Pugh Jr (University of Pennsylvania) and P. G. Polden and J. D. Mollon (University of Cambridge) suggested that a further group of anomalies could be explained if to the second explanatory hypothesis was added a third assumption: an attenuation of the blue-cone signal occurs at the opponent site and this attenuation depends on the difference of the short and long-wavelength inputs, being minimal when the difference is minimal (and when perhaps our sensation is neither blue nor yellow).

With the two assumptions it seemed possible to explain several phenomena discussed in detail at the meeting: the super-additivity of short and long-wavelength fields in reducing the sensitivity of the blue mechanism (Pugh); a newly-reported and paradoxical increase in the sensitivity of the blue mechanism when yellow light is added to a blue adapting field so as to render the composite field brighter but achromatic (Polden and Mollon); the inflexion that is seen in the threshold-*versus*-intensity function for blue targets on long-wavelength fields (W. S. Stiles); apparent saturation of the blue mechanism (Mollon and Polden); and anomalies of light and dark adaptation (Stiles), such as 'transient tritanopia', the strange loss of sensitivity to blue targets that is found after a long-wavelength adapting field has been turned off and that has now been demonstrated at the ganglion cell level by Gouras and Zrenner and at the level of the b-wave of the electroretinogram by D. van Norren (Institute for Perception, Soesterberg).

Other contributors independently provided results pointing to the second and third hypotheses. Thus R. Boynton (University of California), using pairs of stimuli equated for their effects on both red and green cones and therefore discriminated only by blue cones, has found that such discriminations are impaired by red,

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yellow or green fields that have relatively little effect on the blue cones themselves; long-wavelength fields of different wavelength but of the same luminance produce equal losses of discrimination even though their direct effects on the blue cones are very different. M. Alpern and F. Zwas (University of Michigan) have measured the sensitivity of the blue mechanism to adapting beams passing through different points of the pupil and find that the point of entry for best effect is different for short and long wavelength stimuli, suggesting that more than one class of receptor is involved. They add a fresh anomaly to the catalogue: unlike the other cones, the blue cones have their maximal directional and spectral sensitivities at the same wavelength.

Inherited and acquired losses of the blue mechanism may not necessarily result from a loss of blue cones. van Norren demonstrated a blue-cone signal in the electroretinogram of congenital tritanopes; and E. King-Smith, F. Zisman and S. K. Bhargava (University of Manchester) have found that the loss of blue sensitivity in disease is greater for flashes on a yellow field than for flashes on a white field, a result that they relate to the second and third assumptions above.

Lampbrush chromosomes brought up to date

from J. O. Bishop

Nearly 100 years after the discovery of lampbrush chromosomes by Walter Flemming in 1882, what is believed to be the first meeting exclusively devoted to them was held at Maria Laach, in the Eifel in West Germany on May 12–15, 1977. The meeting was arranged by Dr Werner Kunz, University of Düsseldorf, and sponsored by the Deutsche Forschungsgemeinschaft.

DURING the first meiotic division of oogenesis segregation of the chromosomes is suspended, in some organisms for months, after the pairing of homologous bivalents and the formation of chiasmata. The oocyte increases greatly in size and it is reasonable to suppose that, whatever the programme for early embryogenesis, some of its most vital components are laid down at this time. The chromosomes become greatly extended and take up the characteristic lampbrush form in which a chromosome axis, condensed chromomeres and extended lateral loops are all clearly distinguishable in the light microscope.

One feature in particular makes lampbrush chromosomes uniquely interesting. The lateral loops are very actively transcribed. RNA complexed with proteins accumulates on the loops. The form of the RNP complexes and their relationship to the loop vary from one loop to another. This, and the fixed positions of the loops on the axis, makes it possible in some cases to identify the same loop in different oocytes. The RNA products of particular loops can sometimes be identified, and the same loop can be studied at different stages in oogenesis.

Given that a massive increase in the size of the oocyte is necessary, it seems likely that the formation of lampbrush chromosomes is designed to make this possible. The lampbrush form perhaps optimises transcriptional efficiency. On the other hand, since the bivalents are arrested in the process of segregating, perhaps only the parts required for transcription are extended, while the remainder of the chromosome is maintained in a more condensed form. A completely different class of hypotheses is based on the idea that the cell is taking advantage of the growing phase to perform some laborious operation on the chromosomes, such as repair or reconstruction, and that the lampbrush morphology is the result. Of course, these explanations are not mutually exclusive.

It quickly emerged at the meeting that the basis for current thinking about lampbrush chromosomes was laid down between 1950 and 1960, largely due to the work of H. G. Callan in St. Andrews and J. G. Gall at Yale and Minnesota. It was shown that each loop is based on a single extended DNA duplex. When adjacent chromomeres are separated by physical stress, both remain attached to the ends of the intervening loop, suggesting that there is no disruption of the linear continuity of the chromosomal DNA at the junctions of loops and chromomeres. This widely accepted interpretation requires that the pair of chromomeres lying at the base of a loop must be held together by secondary forces. During the 1950s extensive descriptions of lampbrush chromosome sets were published, and genetic polymorphism of loop morphology was discovered. The latter is a strong indication that lampbrush chromosome structure is at least partly determined by the DNA involved.

(from the left) O. Hess, H. G. Callan and E. H. Davidson in discussion.

Transcription

Study of the structure of lampbrush chromosomes is now intimately bound up with inferences as to patterns of transcription. Electron microscope studies of partially deproteinised preparations (O. L. Miller, University of Virginia, Charlottesville) reveal arrays of RNA fibres emanating from a series of polymerase molecules arranged along the loop. In many cases the lengths of the fibres increase regularly along the loop, and this is very reasonably taken to reflect progressive synthesis. Recent autoradiographic studies on *T. c. carnifex* loops that carry histone-specific RNA (see below) are interpreted to show that transcription is initiated before the histone DNA sequences, proceeds through them and continues beyond, with the histone-specifying sequences being cleaved from the transcripts before transcription ends (Callan). Electron microscope studies of loop-associated RNP particles (J. Sommerville, St Andrews) show them to be composed of surprisingly uniform 20-nm particles. The considerably different appearance of the RNP on different loops is apparently due to differences in the quantity and arrangement of these units.

All the evidence available supports the long-standing idea that the lateral loops are transcribed but the chromomeres and the axis are not. Structures with the appearance of transcription complexes are found only on the loops. Antibody to *Drosophila melanogaster* B-form RNA polymerase binds to the loops, but not to the axis or, apparently, to the chromomeres (E. K. F. Bautz; M. Jamrich, Heidelberg).

Provisionally, a transcription unit can be defined as occupying approximately the region between the shortest and the longest fibres observed in an array of transcription complexes (polymerase+fibril). The arrangement of

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Caves as islands

from Robert M. May

FOR exploring questions of competition and of island biogeography, the aquatic fauna of caves offers the advantages that the communities are relatively simple (usually fewer than 10 species) and that many replicates exist. Culver (*Am. Nat.* **110**, 945; 1976) has used these facts in an elegant study of the aquatic communities in caves in river valleys in West Virginia, Tennessee and Virginia. Those who saw the movie *Deliverance* will wonder whether the NSF underwrote his insurance.

Although flatworms, molluscs, crayfish and salamanders are occasionally found, the macroscopic fauna in these caves is dominated by isopods and amphipods (two of the many orders of crustaceans), which account for more than 90% of the numbers and biomass in most caves. The streams in most Appalachian caves consist of a series of short riffles separated by larger pools. All the isopod and amphipod species prefer riffles to pools, partly because the rate of flow of food particles per unit of cross-sectional area in the stream is higher. The domain within a riffle is a patchy environment, with the habitable patches underneath rocks separated by uninhabitable areas where the creatures are exposed to the current. Since all the species are generalised detritus feeders, the main competition is simply for places to avoid the current.

Culver (*Ecology* **54**, 102; 1973) has previously shown how, under these circumstances, the coefficients for interspecific competition can be estimated for laboratory studies using artificial streams. The competition coefficients, α, thus obtained are in good agreement with the observed facts about aquatic cave communities: low α-values are associated with species of different sizes (which tend to be under rocks of different sizes); intermediate α-values are associated with species found in different riffles in the same cave; and species never found in the same cave have high α-values. A more detailed analysis of the data available from the laboratory studies shows that communities with three species should be stable and resistant to invasion, which accords with the field observations.

The discussion, based on competition for habitat, explains many of the features of the aquatic cave communities, and suggests most caves could support three species. The average number of species per cave, is, however, more typically two (2.1 species per cave for the Powell River sites, from a total candidate pool of 4 species, and 2.5 species per cave from Greenbriar River sites, from a total pool of 6 species). Culver attributes this to the caves being, in effect, islands. As a consequence, the average number of species per cave is not simply the maximum number consistent with competitive interactions, but rather is some lesser number set by the interplay between extinction and immigration rates.

Such island biogeographical considerations can be worked out in some detail. The upshot of the processes of immigration and local extinction is that any one cave will tend to have fewer species (typically two), and the entire region to have more species (typically four to six), than would be the case if one had the maximum number of stably coexisting species (typically three) in one large cave. This re-echoes a theme first sounded theoretically by Skellam (*Biometrika* **38**, 196; 1951) and Hutchinson (*Am. Nat.* **95**, 137; 1961), with subsequent elaborations by Horn and MacArthur (*Ecology* **53**, 749; 1972) and by Levins and Culver (*Proc. natn. Acad. Sci. U.S.A.* **68**, 1246; 1971). For organisms of this kind, a conservation programme aimed at preserving maximum species diversity would probably be better served by several small refuges rather than one large one (Simberloff & Abele *Science* **191**, 285; 1976). More generally, the optimum pattern for allocation of conservation areas must depend on the life-history details of the target organism.

Culver concludes by emphasising that aquatic cave communities are basically simpler than terrestrial ones. The available evidence indicates, for terrestrial cave communities, that predation rather than competition may play the dominant role, that seasonal changes are more pronounced, and that the available pool of species is greatly affected by local geography. As Culver puts it: 'If you have seen one [type of] cave community, you have not seen them all.'

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these arrays with respect to the loops varies widely, both within and between species. In many cases a single array occupies all or most of a loop, but some contain a single array which does not span the entire loop, and may occupy as little as 30% (K. E. Scheer, Heidelberg). Other loops contain two arrays, sometimes with the same, and sometimes with opposite polarity. Still others contain multiple arrays—5–10% of the loops in *Triturus*, ~ 50% in the axolotl. When multiple arrays have the same polarity, they may of course represent a single transcription unit with the intervention of one or more processing steps. Where the polarities are opposite this is less likely, and it seems most reasonable to assume for the present that a loop may contain more than one transcription unit.

Organisation

This relates to the relationship between the loops and the axial chromomeres. The proportion of the DNA present in loops is difficult to measure exactly, but it clearly varies widely from one organism to another. In *Triturus* it is put at between 5 and 10%, in *Pleurodeles* and the axolotl at ~50%. At one time it was speculated that each loop is progressively spooled out from one chromomere (the exit strand) and simultaneously gathered up into the other (the re-entrant strand). This is no longer widely believed, and the loops are thought to be more or less constant. The best evidence for this view comes from *Triturus* where it has been shown that the total nuclear RNA corresponds to a transcript of about 5% of the genome—roughly the proportion of the genome found in loops (D. B. Malcolm, St. Andrews). This is not, of course, a rigorous argument.

Techniques have been developed to pick out the same loop or set of loops in different preparations, and in several cases the functions of particular loops have been positively identified. Of the various methods, some depend for their specificity on nucleic acid reassociation, others apparently on the recognition of nucleic acids by proteins, and yet others on antigen–antibody interactions.

Histone genes from a number of echinoderms have been cloned in bacterial plasmids and bacteriophage. Nick-translated cloned DNA has been used to search for the histone genes of *T. c. carnifex* (Callan). Of the five biggish loops in the sexually heteromorphic region of chromosome I, three become labelled with these probes in conditions in which the loop-associated RNA is detected. Four other sets of loops on chromosomes VI(2), X and XI are also labelled. Interestingly, differences were observed between the loop-sets of heterozygous bivalents in two cases, and minor differences between different newts are seen. A loop that is not otherwise visible, presumably because it carries little matrix, is visualised autoradiographically by this technique. What seem to be the same loops can also be labelled (along with others) by using nick-translated middle-repetitive *T. c. carnifex* nuclear DNA, isolated by conventional reassociation methods (H. C. Macgregor, Leicester). The same middle-repetitive fraction of *Triturus* DNA labels six loops near the centromeres of *Plethodon* lampbrush chromosomes perhaps indicating the evolutionary conservation of some families of sequences. A more remarkable conservation is suggested by the labelling of loops in chromosomes II and XI of *Triturus* by human and lungfish DNA (Gall).

Labelled 5S RNA or DNA of *Notophthalmus viridescens* labels loops near the centromeres of chromosomes I, II, VI and VII (P. J. Pukkila, NIMR, London). That of *Triturus c. carnifex* labels a loop on chromosome X. The 5S RNA of the developing oocyte accumulates, together with 4S RNA, in a 40S cytoplasmic RNP particle which predominantly contains only two proteins with molecular weights of 38,000 and 49,000. Antibodies against one of these bind to a loop pair on chromosome X which may be identical to the 5S sequence loop (Sommerville). Antibody against the other binds to several pairs of loops. Antibody raised against a 32,000 dalton protein isolated from the very complex nuclear RNP binds preferentially to 12 pairs of loops. In yet another approach, nick-translated single-stranded DNA binds to two dozen specific loops in conditions that do not favour nucleic acid reassociation, and does so only if RNA has been removed from the preparation (Gall).

Messengers and maturation

Reassociation experiments indicate that the RNA of the oocyte cytoplasm contains only part of the coding potential of the organism. In *Xenopus* the poly(A) RNA of the tadpole contains sequences not found in the oocyte (J. O. Bishop, University of Edinburgh). Similarly, the *Strongylocentrotus* blastula contains mRNA sequences not found in the oocyte (E. H. Davidson, CalTech). On the other hand evidence was presented suggesting that mRNA sequences for both adult and tadpole globin are present at low concentration in *Xenopus* oocytes (M. Rosbash, Brandeis University). It is not really difficult to resolve these results, but we are left without a firm conclusion. On the one side, the reassociation experiments may have failed to detect sequences present in very few copies. On the other, it is possible that sequences with the potential for a very high level of expression also have a relatively high 'leakage' rate when repressed.

Whichever way this question is resolved, it has little direct bearing on the significance of lampbrush loop transcription. Should the oocyte mRNA not contain sequences found elsewhere, those sequences might still be transcribed in the germinal vesicle. A few copies of all mRNAs might reflect a low level of escape synthesis under repression, or synthesis at the derepressed level in an occasional oocyte, rather than synthesis of particular significance.

It is perhaps more profitable to ask to what extent the transcriptional activity of the loops is necessary to account for the RNA present at maturity. Here *Xenopus* and *Strongylocentrotus* are markedly different. A comparison of the poly(A) RNA of stage II (previtellogenic), III and VI (mature) *Xenopus* oocytes by reassociation methods shows them to be qualitatively identical (P. J. Ford, University of Edinburgh). The difference in abundance between the more and less abundant sequences narrows during maturation. Direct measurements indicate that the mRNA synthesised in young oocytes is completely stable. It seems that mRNA is steadily accumulating during maturation, and the synthesis of the more abundant sequences (possibly transcribed from repetitive genes such as histones) is perhaps slowing down. In amphibian oocytes, histone mRNA is largely polyadenylated (M. L. Pardue, Massachusetts Institute of Technology). Calculations based on estimates of the number of active RNA polymerase molecules and the size of the precursor agree that about 3 months would be required to synthesise the number of copies of each sequence found in the mature oocyte (10^6 per oocyte of the less abundant). In *Strongylocentrotus* on the other hand the much smaller complement of RNA can be synthesised in a day, and yet maturation in this organism also takes months (Davidson). A repeated turnover of RNA in the maturing oocyte must therefore occur, which is reflected in the blastula and gastrula: at each stage the bulk of the RNA present is newly transcribed. This applies not only to newly appearing sequences, but also to those common to the previous stage. In *Strongylocentrotus*, therefore, it seems that the accumulation of a store of nascent mRNA for utilisation in early embroyogenesis is not a primary function of the period of oogenesis.

This is not to say that nascent mRNA has no part to play. In both amphibians and echinoderms mRNA is mobilised around the time of fertilisation. It is easy to believe that it plays a part at least in the early cleavage stages. About one third of the oocyte sequences are not found in *Xenopus* tadpoles (Bishop) and in *Strongylocentrotus* two thirds of the sequences found in the oocyte are not found in the pluteus (Hough-Evans).

A hundred years ago

THE next eclipse of the moon is attracting the notice of physicists and aëronauts in France. It is proposed to make a balloon ascent during the night of August 23–24, in order to ascertain whether, independently of clouds, the eclipsed moon has a bloody tinge.

From *Nature* **16**, 16 August, 335; 1877.

Spermatocytes

Spermatocytes also show lampbrush chromosomes. In primary spermatocytes of *Chironomus*, lampbrush chromosomes with thousands of loops are clearly visible in the electron microscope but no transcription complexes are seen (H. G. Keyl, Bochum). In spermatocytes of *Drosophila hydei*, however, the Y chromosome forms at least five pairs of morphogenetically distinctive loop structures that closely resemble oocyte lampbrush loops. Apart from the bobbed loci, Y chromosomes in *Drosophila* carry no classical morphological markers. They do, however, carry genetically defined fertility factors, and these have been closely studied in *D. hydei* (O. Hess, University of Düsseldorf). A genetic deficiency of one or another loop is always accompanied by failure of fertility. Some years ago, Hennig (*J. molec. Biol.* **38**, 227; 1968) showed that the spermatocytes contain Y chromosome-specific RNA. Also, treatment with actinomycin D causes regression of the loops. In spread preparations transcription complexes are observed (K. H. Glätzer, Düsseldorf). One pair of loops, however, called threads, shows some peculiarities. The pattern of uridine incorporation, the binding sites of RNA polymerase antibodies, and the matrix morphology suggest that the distal parts of the threads are not active in transcription but seem to function as RNP storage organelles. But other explanations are clearly possible.

The relationship of the spermatocyte lampbrush Y chromosome to oocyte lampbrushes is not all clear. However, this system is most promising in its own right, both because of the localised effect of the fertility factors and because a truly cytogenetic approach is clearly possible. ☐

review article

Taxonomy and the origin and evolution of cultivated plants in the New World

Barbara Pickersgill*

Current taxonomic problems in cultivated plants include delimitation of species, whether speciation has occurred under domestication, and the role of weedy relatives in the evolution of domesticates. Many cultivated plants of the New World seem to have been domesticated not once but several times, and agriculture itself may well have originated independently in four different parts of the Americas.

STUDIES of the origin and evolution of cultivated plants aim to answer four basic questions: from what did our modern crop plants originate; where were they domesticated; when did this happen; and how have these crops changed and spread since they were first taken into cultivation? In 1886, Alphonse de Candolle[1] outlined the pertinent sources of data in order of usefulness: first, archaeology; second, botany; third, history; fourth, philology. Today all these types of evidence are still used, though 'botany' in de Candolle's sense has become the province of taxonomy in particular, and involves more than gross morphology and geographical distribution. Cytotaxonomic studies are now routine, while chemotaxonomy may furnish additional information on relationships. New micromorphological characters have become available with the development of the scanning electron microscope and, together with improved archaeological techniques for the recovery of plant material, are greatly assisting identification and analysis of archaeobotanical specimens. Numerical taxonomic techniques enable taxonomists to analyse variability and assess resemblances using many variables, from many different fields, simultaneously.

Species concepts in cultivated plants

When investigating the origin(s) of a particular crop, the first essential is to know how many species are included in that crop. Although Darwin[2] and de Candolle[1] called attention to the striking and discontinuous intraspecific variation characteristic of cultivated plants, taxonomists of the period often elevated these variants to the rank of species. With the rediscovery of Mendelian genetics, many of these 'species' were correctly evaluated as simply lines differing in one or a few major genes. The development of genecology focussed attention on the process of speciation, particularly the role of reproductive isolation in the differentiation of species. This likewise led to the recognition of fewer, but more variable, species. The taxonomist's task is to distinguish between discontinuities which may seem very striking but which nevertheless represent simply major gene variants within a cultivated species, and discontinuities which may appear trivial but which delimit boundaries between species.

The effect of these changing species concepts is well illustrated by the chili peppers (*Capsicum*). In the nineteenth century, over 60 species were described, differing mostly in colour, size, shape and pungency of the fruit. In 1923, Bailey[3] reduced these to a single species. Now, five different species of domesticated chili pepper are recognised[4], mainly by floral characters, since the more strikingly discontinuous fruit characters show parallel variation within the domesticated species.

Speciation and domestication

Improved taxonomic understanding of other crops has shown that not only *Capsicum*, but also cotton, tobacco, beans, squash and amaranth, among others, comprise two or more cultivated species, grown for the same product and used in the same way, but usually with their own characteristic geographical distributions[5]. This poses the problem of whether speciation preceded domestication (which implies that the individual cultivated species were domesticated independently, from wild ancestors which were already specifically distinct), or whether speciation followed domestication (in which case each crop may have been domesticated once only). This question has to be answered separately for each crop, and the answers may well be different.

In cotton, truly wild plants (not escapes) are known in both Mesoamerican *Gossypium hirsutum* and South American *G. barbadense*. The earliest archaeological cotton from South America belongs to *G. barbadense* and seems to represent a very early stage in domestication[6]. The earliest Mexican archaeological cotton is, however, 'almost certainly' *G. hirsutum* and is fully domesticated, although it is older than the earliest Peruvian specimens[7]. These data strongly suggest independent domestication of the two cultivated species of New World cotton from specifically distinct wild ancestors.

Among South American cultivated diploid potatoes, on the other hand, four species have been recognised[8]. *Solanum stenotomum* is the basic, and most variable, species. *Solanum goniocalyx* is a northern derivative which consists of a few relatively invariable clones. *Solanum ajanhuiri* probably results from hybridisation between cultivated *S. stenotomum* and the wild frost-resistant species, *S. megistacrolobum*[9]. *Solanum phureja*, adapted to the wet eastern slopes of the Andes and distinguished by early maturity and non-dormant tubers, was perhaps derived by assortative mating within the original variable cultivated complex[10]. Here, domestication has been followed by increasing differentiation, to the verge of speciation, within the cultivated populations.

Chili peppers present a more complex situation. Of the five domesticated species, *Capsicum baccatum* and *C. pubescens* are morphologically and cytogenetically so distinct that they must each have been domesticated independently from distinct wild species.

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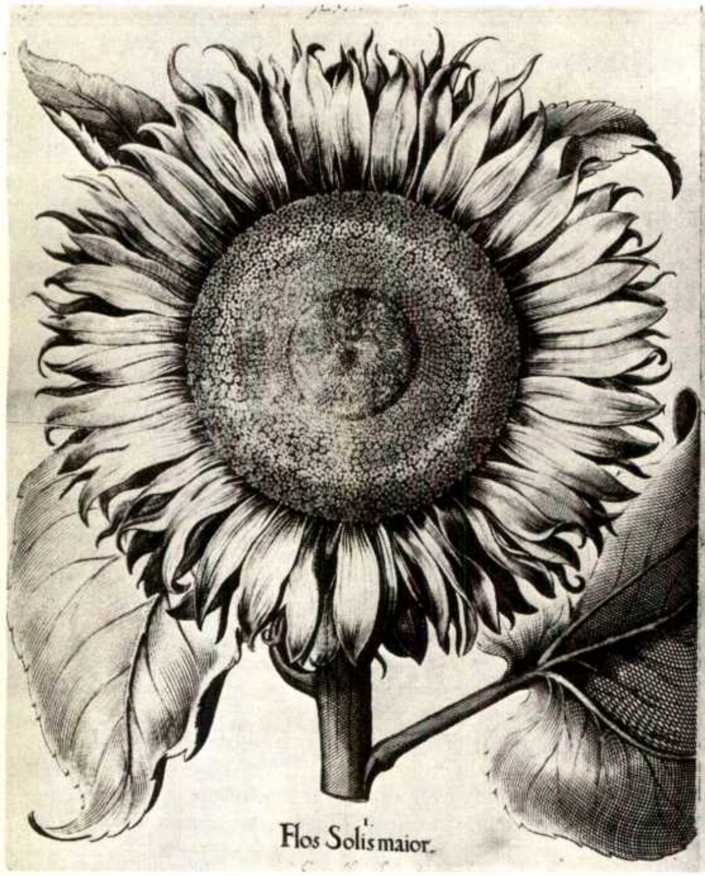

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Fig. 1 *Helianthemum*. From *Hortus Eystettensis*, vol II, Besler, 1613.

II.
Serpillum citratum.
I.
Papas Peruanorum.
III.
Thymus vulgatior.

Fig. 2 *Solanum*. From *Hortus Eystettensis*, vol II, Besler, 1613.

Numerical taxonomic studies[11] confirm the probable independent domestication of *C. baccatum* but suggest that domesticated *C. annuum*, *C. chinense* and *C. frutescens* all arose from different parts of the same incompletely differentiated complex of wild forms. In chili peppers, therefore, speciation in some instances preceded, and in other instances was accentuated by, domestication.

The role of weedy relatives

Often the closest wild relatives of a cultivated plant prove to be weedy forms in the same, or a closely related, species. As de Candolle[1] noted, distinguishing genuinely wild ancestors from feral descendants of a crop is often very difficult. Again, the problem must be evaluated independently for each crop and the decisive evidence may come from very different sources.

In cotton[12], primitively wild forms have small bolls, often with sharp recurved beaks, and greyish-white or dingy brown lint sparsely covering the small hard seeds, which are dispersed individually. The beak has been reduced in size in hand-harvested domesticated cottons and has not been regained in feral cottons. Feral cottons also retain the clear white or uniformly brown lint of their cultivated ancestors and their lint is usually so abundant that all seeds in one locule of the boll are dispersed as a unit. Critical analysis of morphological features may thus permit primitively wild cottons to be distinguished from escaped feral forms.

In *Cucurbita*[13], there has been argument over whether weedy *C. andreana* is the wild ancestor or an escaped derivative of the South American cultivated *C. maxima*. With the exception of *C. ecuadorensis*, whose status also seems doubtful, no other wild species of *Cucurbita* is known from South America. *Cucurbita* flowers are pollinated by various insects, including squash bees *(Peponapis)* which are wholly dependent, as larvae and adults, on *Cucurbita* flowers for food. Three species, representing three different subgenera of *Peponapis*, are confined to South America. It seems unlikely that South American *Peponapis* could have diverged as much as this if *Cucurbita* had been introduced by man (hence comparatively recently) from Middle to South America. Here, then, taxonomic study of the pollinator suggests an ancient presence of more than one species of *Cucurbita* in South America, presumably including *C. andreana*, hitherto considered only doubtfully wild.

Many wild chili peppers also occur in weedy contexts, raising doubts about their relationships to the domesticates. Virtually all accessions of domesticated *Capsicum annuum* belong to the same, derived, chromosome race, which in wild *C. annuum* occurs only in Mexico[14]. Other wild forms of *C. annuum* carry the basic karyotype shared by most species in the genus. Cytological data thus strongly suggest that weedy forms of *C. annuum* are primitively wild and that only the Mexican weedy forms are involved in the origin of domesticated *C. annuum*.

Electrophoretic studies on isozyme variability may also help in unravelling relationships between cultivated and weedy races. The New World cultivated chenopods include one tetraploid, *Chenopodium nuttalliae*, in Mexico and one tetraploid, *C. quinoa*, in the Andes. The two are very similar and there has been some doubt as to whether they really represent distinct species. Weedy tetraploids also occur in the Andes *(C. hircinum)* and from Mexico north into North America *(C. berlandieri)*. All the tetraploids carry two unlinked genes for synthesis of the enzyme leucine aminopeptidase (LAP) (ref. 15). Virtually all Andean accessions of cultivated *C. quinoa* and weedy *C. hircinum* are homozygous for *Lap-A2* and *Lap-B2*. Mexican cultivated *C. nuttalliae* and weedy *C. berlandieri* are also homozygous for *Lap-A2* but at the *Lap-B* locus they carry *Lap-B1* not *Lap-B2*. In North America, outside the range of *C. nuttalliae*, *C. berlandieri* is polymorphic for *Lap-A* and monomorphic for *Lap-B2*. These studies support the view that *C. nuttalliae* and *C. quinoa* are distinct species; fit with earlier data on genetic control of fruit colour which imply that pale-fruited domesticated forms of *C. nuttalliae* and *C. quinoa* originated independently[16]; and suggest that weedy *C. berlandieri*, at least, is more likely to be ancestral to its related cultigen than derived from it.

1 Cucurbita lagenaria ſylueſtris.
Wilde Bottle Gourd.

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Fig. 3 Bottle gourd. From the *Herball* 2nd edn Gerarde, 1636.

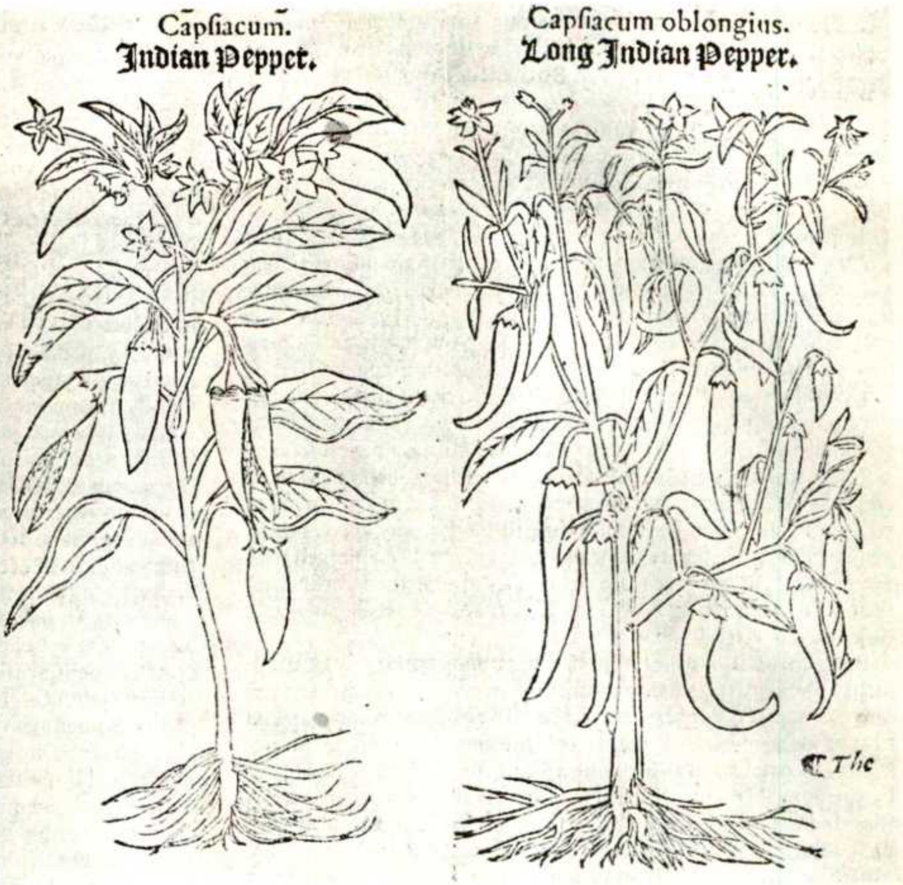
Capſiacum.
Indian Pepper.
Capſiacum oblongius.
Long Indian Pepper.
The

Fig. 4 *Capsicum*. From *Theatrum Botanicum*, Parkinson, 1640.

Origins of polyploid crops

Many New World cultivated plants are polyploids and here the direction of evolution, from diploid to polyploid, is unequivocal. The initial steps in determining the ancestry of a polyploid are now largely routine, but the results of these investigations are interpreted more cautiously than was the case a decade or so ago. The potato has many attributes of an autotetraploid, with four homologous sets of chromosomes, but forms mainly bivalents at meiosis[17], that is, shows pairing behaviour usually associated with allopolyploidy. Cotton is a classic allotetraploid, but in AD polyhaploids derived from the AADD cultivated cottons there is less pairing between A and D chromosomes than occurs in newly synthesised AD hybrids[18]. This suggests a genetic restriction of pairing between A and D chromosomes which exaggerates the apparent difference between the two genomes. Chromosome pairing alone may thus be an unreliable guide to the nature and origin of a polyploid.

Comparative morphology may also be misleading. The most probable ancestor of the cultivated tetraploid potato (*Solanum tuberosum*) is the cultivated diploid *S. stenotomum*. *Solanum stenotomum* has, however, an irregular bilabiate calyx, while the calyx of *S. tuberosum* is always regular. The irregular calyx of *S. stenotomum* is recessive to the regular calyx of the weedy diploid *S. sparsipilum*, and Hawkes[8] has postulated that tetraploid *S. tuberosum* arose by chromosome doubling in a hybrid between *S. stenotomum* and *S. sparsipilum*. Apparent confirmation of this parentage has come from serological and electrophoretic studies[19], as well as observations on polyhaploids of *S. tuberosum*[20], some of which have an irregular, others a regular, calyx.

Different lines of investigation sometimes suggest different conclusions. *Nicotiana tabacum* is an allotetraploid combining the S genome from *N. sylvestris* and the T genome from one of the four species in section Tomentosae. Geographic distribution and fertility of synthesised amphidiploids suggest the T genome donor was *N. otophora*, while morphology of the synthesised amphidiploids, genetic segregation ratios in hexaploid hybrids between *N. tabacum* and the T genome diploids, and chemotaxonomic studies on sterol composition and isozyme variation all implicate *N. tomentosiformis* rather than *N. otophora*[21,22]. A possible explanation of these discrepancies is that the T genome of *N. tabacum* was not derived from any of the extant species but from an extinct progenitor common to both *N. tomentosiformis* and *N. otophora*, though this raises a further problem since *N. tabacum* is unknown in the wild and is usually assumed to have originated in cultivation, that is, fairly recently on the evolutionary time scale.

The question of multiple origins arises again in polyploid crops. If their ancestral diploids were widespread, hybridisation, chromosome doubling and successful establishment of the resultant polyploids could have occurred more than once and in more than one place. For example, a polyphyletic origin has been postulated, on chemotaxonomic grounds, for the tetraploid New World cottons. Johnson[23] argued, from seed protein electrophoretic patterns, that *Gossypium hirsutum* arose from hybridisation between an AA diploid and two different DD diploids, while studies of floral pigments[24] may imply that different DD diploids were involved in the origins of *G. hirsutum* and *G. barbadense* respectively. These two interpretations are in part mutually contradictory, and do not agree with cytogenetic evidence that the D genomes of *G. hirsutum* and *G. barbadense* are identical[25]. Nevertheless, polyphyletic origins must be borne in mind when polyploid crops are considered.

Taxonomy and the domestication and dispersal of cultivated plants

Although the archaeological record potentially provides the most conclusive evidence on where a particular crop was domesticated, this record is so incomplete that inferences often have to be drawn instead from the present distribution of the crop and its wild relatives. Criteria usually used to determine the species most closely related to a crop are morphological similarity and ability to hybridise with the crop. However, in some groups, such as *Manihot*, speciation has not been accompanied by reproductive isolation so this latter criterion is useless. Rogers and Appan[26] resorted to numerical taxonomic analyses in an attempt to determine where and from which species cultivated manioc (*M. esculenta*) was derived. They found that species morphologically close to *M. esculenta* occur in both Middle and South America, so were unable to decide in which continent *M. esculenta* arose.

Improved taxonomic understanding may radically alter views on the wild relatives and hence place of origin of particular cultivated plants. The coconut (*Cocos nucifera*)[27] was once thought to have originated in the New World because this was where the other species of *Cocos* occurred. Now, however, *Cocos* is treated as a monotypic genus whose closest living relative is African. This, together with the fossil record of the coconut and its variability and range of uses in south-east Asia, suggests that the coconut originated in the western Pacific and spread west to east, not east to west, across the ocean. The bottle gourd (*Lagenaria siceraria*) is another pantropical cultivated plant unknown in the wild and considered for a long time to be the only species in its genus. Now, however, two African genera have been merged with *Lagenaria*[28], in part because they readily give fertile hybrids with the bottle gourd, and it now seems that *L. siceraria* itself may have originated in Africa.

Both coconut and bottle gourd have figured largely in arguments about prehistoric human trans-oceanic voyages. Archaeologists and geographers tend to be struck by the coincidence of similar uses of similar plants on different sides of an ocean; botanists, aware that these same disjunct distributions are shared by various wild plants which are no conceivable use to man, are not found in man-disturbed habitats, and are hence most unlikely to have been spread either accidentally or intentionally by man, are usually less impressed. Seventy-four genera, including 108 species, are common to tropical America and tropical West Africa; 94 genera occur in both tropical South America and tropical Asia–Australasia[29]. Many of the species involved are far less well-adapted for natural long-distance dispersal than the coconut or the bottle gourd.

Centres of diversity and centres of domestication

Vavilov[30] employed the argument that centre of diversity is a guide to centre of domestication extensively in his studies on the origin of cultivated plants, though he was careful to distinguish primary centres, where diversity is a consequence of ancient cultivation, from secondary centres, where diversity may be a consequence of hybridisation. Later critics of Vavilov's work have suggested that ecological diversity[31], farming practice[32], human migration patterns[33] and/or the breeding system of the crop[34] could likewise produce localised centres of diversity, in which diversity would however be unrelated to the length of time for which the crop had been cultivated. Some cultivated plants, such as the bottle gourd, may have no centre of diversity, but were probably domesticated repeatedly throughout the range of their wild progenitors[35]. Despite the availability of appropriate techniques—notably isozyme analysis—for comparative studies of variation, and despite the interest among those concerned with conservation of crop genetic resources in knowing more about distribution of variability within crops as a guide to exploration and sampling, there have been few thorough biosystematic investigations of cultivated plants inside and outside the range of their wild relatives[36,37] and it remains difficult to determine how generally applicable Vavilov's criteria are for determining the place of domestication of a cultivated plant.

Maize is a classic example of a crop in which variability may give misleading evidence on place of domestication. The centre of diversity of maize is in Peru, yet archaeological evidence and distribution of wild relatives demonstrate conclusively that maize was domesticated in Mexico[38]. In tomato also the centre of diversity of the cultigen (Mexico) and the distribution of related wild species (western South America) do not coincide. Here archaeological, historical and linguistic data indicate that Mexico was the centre of domestication[39].

Taxonomy and the archaeological record

Interpretation of the archaeological record of cultivated plants is very much the province of taxonomy. The first step is identification and, because seeds and other hard plant parts are more likely to be preserved than fleshy fruits, flowers or unlignified vegetative organs, identification usually cannot be based on traditional taxonomic diagnostic characters but has to rely instead on such characters as are available. Scanning electron microscope studies of surface features of carrot mericarps from late, possibly post-Spanish, levels of a site in coastal Peru showed that these fruits belonged to one of the indigenous New World wild species, not the introduced Mediterranean *Daucus carota* (B.P., unpublished, and S. Okeke, personal communication). Similarly, scanning electron microscope studies of starch grains and vascular tissue, particularly xylem, seem to offer the best hope of identifying various starchy tubers and roots from other Peruvian sites[40]. Phytoliths (siliceous bodies in the epidermal cells of many grasses, whose shape may differ in different genera) may be preserved archaeologically when most other plant parts have decayed and attempts are being made to use phytoliths to trace the spread of maize cultivation from Mexico to South America (D. M. Pearsall, papers delivered at the 41st and 42nd Annual Meetings of the Society for American Archaeology, 1976 and 1977), through humid tropical regions where archaeobotanical preservation is generally poor.

After identification, the next step is to decide whether the archaeobotanical material comes from wild or domesticated plants. Sometimes domestication has been accompanied by morphological changes that can be recognised in archaeological specimens. In *Phaseolus vulgaris*[41], both the parchment layer and the fibrous thickening of the sutures of the pods have been reduced, producing tardily dehiscent or indehiscent pods in the cultigens. The structure of the seed coat has also altered so that seeds of domesticated beans are more permeable, hence germinate more rapidly. In chili peppers[42], natural mechanisms for seed dispersal have likewise been lost, and fruits of domesticated peppers are retained on the peduncle at maturity.

In many crops domestication has not been accompanied by qualitative changes of a sort that could be detected archaeologically. Quantitative changes in size have been extensively used in studies of animal domestication but are treated more cautiously by botanists. Exploitation, cultivation and domestication of avocadoes have been distinguished at different periods of a long archaeological sequence from Mexico[43], but this interpretation has been criticised because both parameters used (numbers of seeds, and size of largest seed) are themselves in part related to time[44]. Archaeological fruits of sunflower, *Chenopodium* and *Iva* from sites in eastern North America which antedate the spread of maize agriculture from Mexico are larger than fruits of modern weedy forms. Those who do not believe that agriculture was invented independently by the Eastern Indians have argued that growth on nutrient-rich soils such as middens, selective harvesting and/or post-harvest processing could lead to fruits recovered from archaeological contexts being outside the size range of modern wild fruits; proponents of the hypothesis have pointed out that other weedy plants, such as *Phalaris*, which were collected but not cultivated, do not have larger fruits than modern forms[45]. *Iva* has in addition been found archaeologically east of its present range as a wild plant, suggesting that man was involved in the spread of this species[46].

Agricultural origins in the Americas

De Candolle[1] considered that agriculture had originated independently in three regions: China; the Middle East and Egypt; and tropical America. Vavilov[30] recognised eight centres of origin of cultivated plants, including two in the New World. More recent taxonomic studies of New World cultivated plants and collaboration between taxonomists and archaeologists in interpreting evidence on their domestication, evolution and spread suggest that agriculture may have developed essentially independently in four different parts of the Americas. In Mesoamerica and the Andes the first unequivocal domesticates appear about the same time, 5500–5000 BC (ref. 46), but the plants cultivated in these two centres appear in a different sequence and often belong to different species. Later some crops, notably maize, were exchanged between the two areas, but the introduced crops were absorbed into the indigenous agricultural complexes without altering the essentially distinctive character of the two regions. A third complex of crops was domesticated in the humid lowland tropics, but it is difficult to determine how early agriculture began here because conditions seldom favour preservation of identifiable archaeobotanical materials. Lowland domesticates which could survive in semi-arid conditions (manioc, sweet potato, peanut, pineapple) spread to the Mesoamerican and Andean centres, while crops which could adapt to high rainfall (maize, cotton) spread down into the jungle lowlands. In eastern North America a fourth complex of cultivated plants may have developed, but this was almost completely supplanted by the northwards spread of the Mesoamerican domesticates.

Multiple origins thus seem likely for New World agriculture in general as well as for most of those New World crops which include more than one species. Further and more critical taxonomic and archaeological work may well demonstrate that even single-species crops may have been domesticated repeatedly within the range of their wild progenitors. Domestication of cultivated plants no longer seems to be as unique and difficult a process as was once thought.

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articles

Oceanic residence times of trace metals

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A detailed study of suspended matter and metal fluxes in a large marginal sea has enabled us to assess the impact of nearshore precipitation of the dissolved and particulate constituents of river discharge upon the oceanic residence times of seven trace metals. A distinct improvement in the agreement between stream flow and sedimentation rate estimates of residence time has been achieved and we conclude that most previous determinations, particularly in the cases of Fe and Mn, have been overestimated. Our calculations yield oceanic residence times of Fe which are compatible with direct measurements of the settling rate of Fe in the deep ocean.

THE oceanic residence times of trace metals have most commonly been estimated by determining either the rates of metal injection to the ocean from rivers (the stream-flow method) or the rates of metal precipitation into oceanic sediments. In a majority of cases, the metal concentrations used in these calculations to determine the standing stock of metals in the ocean have not been the most recent and reliable estimates. This has generally led to overestimates of residence time irrespective of which method has been used. In addition, estimates based on stream-flow have largely neglected both nearshore precipitation of metals and the discharge of metals to the ocean in riverborne suspended particulate material. Obviously, for metals that are predominantly in solution in rivers, and which do not undergo appreciable precipitation in estuaries, neither of these considerations will seriously alter the calculated residence times. For elements such as Fe, however, which significantly precipitate in estuaries[1–4], residence times calculated without accounting for this removal will be underestimated. Conversely, although most riverborne particulates undoubtedly precipitate in the nearshore zone, the residual particulate flux will contribute large amounts of some metals, for example, Fe and Mn to the ocean. If these fluxes are neglected, stream flow residence times will be overestimated.

It may be argued that such biases would be revealed by a comparison of the rate of incorporation in sediments with the rate

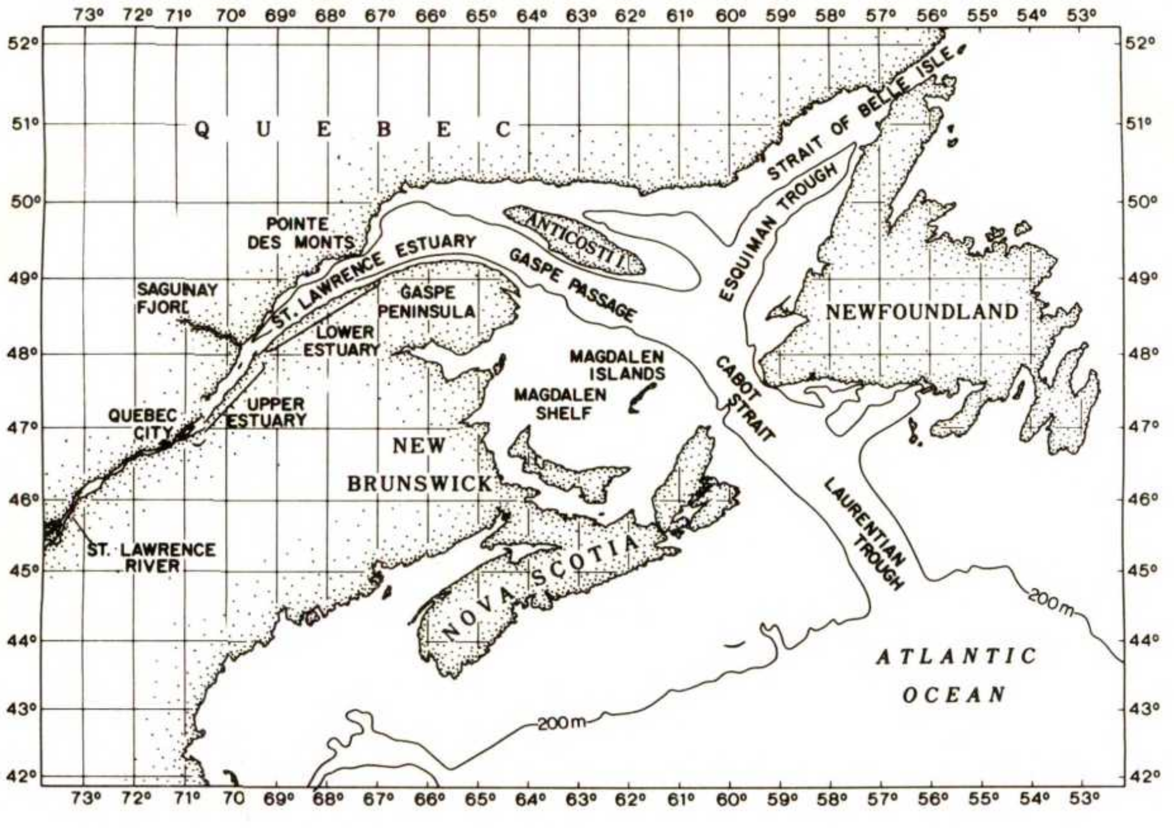

Fig. 1 Estuary and Gulf of St Lawrence.

of injection from rivers. We seek to show that this is indeed the case, and that taking into account (1) the extent of nearshore metal precipitation and (2) the particulate as well as the dissolved, river discharges of each metal, results in improved agreement between stream flow and sedimentation rate estimates of oceanic residence times. The use of recent values for oceanic trace metal concentrations should also improve the accuracy of these estimates.

This paper is based on data collected on chemical oceanographic surveys of the St Lawrence river, estuary and Gulf of St Lawrence, the Saguenay Fjord, and the western North Atlantic during the period 1972–1976. The estuary and Gulf of St Lawrence (Fig. 1) comprise a large marginal sea on the eastern seaboard of North America. Of the freshwater input to the entire system, 71% is derived from the St Lawrence River which is the eleventh largest world river in terms of water discharge. We have determined the concentrations of suspended particulate matter (SPM) and seven metals in inflowing fresh and saline waters and outflowing mixed water at the two extremities of the system (Quebec City and Cabot Strait) and at two other sections in the estuarine region (near the entrance to the Saguenay Fjord and at Pointe des Monts). Nuclepore filters (0.4 μm pore size) were used for the gravimetric determination of SPM concentrations and the metals were analysed in unfiltered samples (Fe, Mn, Co, Ni, Cu, Zn, Cd) and filtered samples (Fe, Mn) by flameless atomic absorption techniques. The resultant data have been used in a mass and salt conservation model (Fig. 2) to calculate the fluxes of each metal flowing into and out of three sequential regions of the entire marginal sea (Table 1). Since these data probably only pertain to the instantaneous fluxes at the time of sample collection, it would be unrealistic to use them for estimating the integrated annual fluxes at each section. We have only assumed, therefore, that the proportions of each component precipitated, or otherwise removed, within each region of the system remain unaltered as stream flows change with season. It has also been assumed that direct atmospheric precipitation of particulates and trace metals into the system is negligible and that exchanges through the Strait of Belle Isle, which, for water, are about 1% of those at Cabot Strait, can be ignored. In the former respect, particulate atmospheric deposition has been estimated to be about 1% of the overall freshwater particulate input[6]. Finally, since the St Lawrence River is unlikely to be the sole source of sediments within the system, we have assumed that, for each metal, the percentage losses from all freshwater and marine sources are identical. As an independent assessment of the validity of the calculation we also compare in Table 1 the concentration of each metal in the internally precipitating material with the composition of pelitic sediments within the Gulf. The comparison is surprisingly good. Similarly, the calculated sedimentation rate of 415 kg s^{-1} (1.3 × 10^{7} t yr^{-1}) agrees well with Sundby's[6] estimate (1.0 × 10^{7} t yr^{-1}) of the rate at which fine-grained sediments have accumulated during the present circulation regime.

The results of scaling the St Lawrence data to the global situation are illustrated in Table 2. We first determined the weighted average composition of St Lawrence River water based

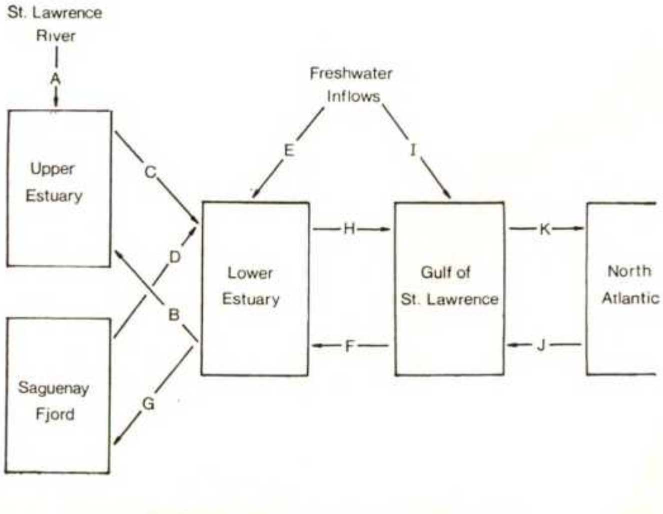

Fig. 2 Model of the St Lawrence system.

Table 1 Fluxes of suspended matter and metals through the St Lawrence system

Region		Flux	Units	Water	SPM	Fe_d	Fe_p	Mn_d	Mn_p	Co_t	Ni_t	Cu_t	Zn_t	Cd_t
Upper Estuary	A	St Lawrence River inflow	kg s^{-1}	$2.1\ 10^{7}$	336	1.16	24.0	0.202	0.407	0.0085	0.040	0.105	0.162	0.0074
	B	Saline counter-current inflow	kg s^{-1}	$5.6\ 10^{7}$	120	0.78	10.3	0.088	0.139	0.0050	0.040	0.045	0.082	0.0126
	C	Mixed outflow	kg s^{-1}	$7.7\ 10^{7}$	454	1.37	30.4	0.333	0.3405	0.0098	0.090	0.114	0.137	0.0190
	A+B−C	Loss	kg s^{-1}	—	2	0.57	3.9	−0.043	0.2055	0.0037	−0.010	0.036	0.107	0.0010
Lower Estuary	D	Saguenay Fjord inflow	kg s^{-1}	$4.4\ 10^{6}$	26.1	0.31	2.59	0.048	0.026	0.0004	0.003	0.004	0.009	0.0006
	E	Additional freshwater inflow	kg s^{-1}	$2.7\ 10^{6}$	27.0	0.14	2.57	0.027	0.054	0.0008	0.004	0.011	0.027	0.0008
	F	Counter-current inflow	kg s^{-1}	$9.24\ 10^{7}$	20.5	0.345	0.825	0.060	0.011	0.0018	0.032	0.059	0.196	0.0117
	G	Counter-current outflow to the Saguenay Fjord	kg s^{-1}	$2.2\ 10^{6}$	5	0.03	0.3	0.004	0.005	0.0002	0.001	0.001	0.003	0.0005
	H	Mixed outflow	kg s^{-1}	$1.18\ 10^{8}$	248	0.95	14.08	0.324	0.175	0.0049	0.065	0.130	0.291	0.0150
	(C+D+E+F) −(B+G+H)	Loss	kg s^{-1}	—	154	0.40	11.71	0.052	0.112	0.0027	0.023	0.012	−0.007	0.0040
Gulf	I	Additional freshwater inflow	kg s^{-1}	$3.7\ 10^{6}$	37	0.185	3.515	0.037	0.074	0.0011	0.006	0.015	0.038	0.0011
	J	Counter-current inflow	kg s^{-1}	$3.82\ 10^{8}$	19.4	0.930	1.526	0.148	0.0454	0.0131	0.171	0.223	0.581	0.0194
	K	Mixed outflow	kg s^{-1}	$4.12\ 10^{8}$	24.5	1.24	2.320	0.499	0.0603	0.0177	0.197	0.317	0.808	0.0245
	(H+I+J) −(F+K)	Loss	kg s^{-1}	—	259	0.48	15.98	−0.050	0.223	−0.0004	0.013	−0.008	−0.094	−0.0007
		Sum of losses	kg s^{-1}	—	415	1.45	31.59	−0.041	0.541	0.0060	0.026	0.040	0.006	0.0043
Whole System		Metal concentration in internally sedimenting material	%	—	—	8.0		0.13		0.0015	0.0063	0.0097	0.0015	0.0010
		Metal concentration in Gulf of St Lawrence pelitic sediments (from refs 18 and 19)	%	—	—	6.0		0.10		0.0016	0.0033	0.0025	0.0100	0.00002
		Proportion of total inputs lost internally	%	—	93	89		47		25	12	11	0.1	15

Subscripts d, p and t denote dissolved, particulate and total metal respectively.
Characterisation of water flows in Cabot Strait and Pte. des Monts sections are based upon the geostrophic calculations of El-Sabh[20].

on duplicate samples collected at two stations on a section across the river at Quebec City for the months April to September inclusive. We then calculated the resulting river-derived annual fluxes through Cabot Strait by correcting for the deposition of the river constituents within the entire marginal sea. Estimates of the particulate fraction of each metal in the Cabot Strait outflow were then used to split the total fluxes into dissolved and particulate components. For Fe and Mn, these estimates are based upon experimental determinations while others are calculated from average terrigenous rock composition and the concentrations of total metal and suspended matter in the outflow. The dissolved and particulate metal fluxes have then been scaled up to the world river discharge on the assumption that the losses of river-derived material in the Gulf of St Lawrence are typical of those in the nearshore receiving waters of all world rivers. The scaling has been achieved by using Holeman's[7] figure for the particulate material discharge via rivers to scale the fluxes of particulate metal and Livingstone's[8] value for the world river water runoff to scale the fluxes of dissolved metal. Implicit in the former scaling process is the assumption that the composition of particulate material discharged by other rivers is similar to that found in the case of the St Lawrence. Since the composition of weathered terrigenous rock is not greatly variant, especially in the cases of Fe and Mn, this assumption is not unreasonable. Similarly implicit in the dissolved metal scaling process is the assumption that the dissolved composition of St Lawrence River water is typical of that of world rivers. Obviously, the validity of this assumption must await more accurate estimates of the composition of other major river waters but the values we have used are well within the range of commonly accepted values for other fresh water discharges.

Having carried out this scaling process it is a simple matter to recombine the dissolved and particulate metal fractions and calculate the oceanic residence times using 1.32×10^{21} *l* as the volume of water within the oceanic basins and the average concentration of each metal in oceanic waters shown in Table 2, after Bewers *et al.*[9]. Comparisons between these latter concentrations and other previously reported values are made in this reference. The agreement with values reported more recently[10–12] is good. The results of such a calculation and a comparison with residence time estimates based on those of Goldberg and Arrenhius[13] but using the recent oceanic metal concentrations are also shown in Table 2.

We have not attempted to calculate the residence time of particulate material since this requires a better knowledge of the mean concentration of purely detrital material in the deep ocean. Nevertheless, we have included in Table 2 an estimate of the suspended particulate influx to the ocean although we realise that its acceptance implies that there is no substantial export of internally generated biogenic particles through Cabot Strait. If the terrigenous detrital component of particulate material is diluted with internally generated biogenic particles the nearshore losses of suspended matter will be underestimated. Our estimates of particulate fluxes at Cabot Strait are, however, based on measurements in July 1976 at which time the composition and quantity of suspended matter in the Gulf of St Lawrence outflow indicate that the material is predominantly detrital.

Table 2 Residence time calculations

	Units	SPM	Fe	Mn	Co	Ni	Cu	Zn	Cd
Annual outflow from St Lawrence River	kg yr^{-1}	4.4×10^{9}	3.8×10^{8}	1.0×10^{7}	1.4×10^{5}	7.6×10^{5}	1.4×10^{6}	2.2×10^{6}	1.2×10^{5}
River flux corrected for nearshore losses	kg yr^{-1}	3.08×10^{8}	4.18×10^{7}	5.30×10^{6}	1.05×10^{5}	6.69×10^{5}	1.25×10^{6}	2.20×10^{6}	1.02×10^{5}
Particulate fraction at Cabot Strait	%	100	65	11	4	1.3	0.7	0.2	0.02
Global dissolved flux	kg yr^{-1}	—	1.08×10^{9}	3.48×10^{8}	7.42×10^{6}	4.86×10^{7}	9.11×10^{7}	1.62×10^{8}	7.51×10^{6}
Global particulate flux	kg yr^{-1}	1.26×10^{12}	1.11×10^{11}	2.39×10^{9}	1.72×10^{7}	3.56×10^{7}	3.57×10^{7}	1.80×10^{7}	8.35×10^{4}
Sum of global dissolved and particulate fluxes	kg yr^{1}	1.26×10^{12}	1.12×10^{11}	2.74×10^{9}	2.46×10^{7}	8.42×10^{7}	1.27×10^{8}	1.80×10^{8}	7.59×10^{6}
Concentration in marine waters (ref. 9)	$\mu g\ l^{-1}$	—	2.27	0.08	0.023	0.23	0.39	1.07	0.044
Oceanic residence time based on stream flow	yr	—	27	39	1200	3600	4100	7800	7700
Oceanic sedimentation rate (from ref.13)	kg yr^{-1}	1.5×10^{12}	1.0×10^{11}	2.0×10^{9}	3.9×10^{7}	1.7×10^{8}	8.0×10^{7}	7.8×10^{7}	6.3×10^{5}*
Oceanic residence time based on sedimentation rate	yr	—	30	53	780	1800	6400	18000	92000

*Assumed mean Cd concentration in ocean sediments of 0.42 mg kg^{-1} (ref. 21)
Global dissolved flux based on scaling river water discharge to world river runoff of 3.24×10^{16} kg yr^{-1} (ref. 8)
Global particulate flux based on scaling river particulate discharge to world river sediment discharge of 1.8×10^{13} kg yr^{1} (ref. 7)
Use of three significant figures in intermediate calculations is merely to avoid roundoff errors in tabulated values.

As can be seen in Table 2, the agreement between the trace metal residence times based on stream flow and sedimentation rate is, with the exception of those for Cd, quite good and certainly better than other recent comparisons[10,14]. Furthermore, the calculated influx of fluvial suspended particulate matter is comparable with the rate of oceanic sedimentation[13]. The discrepancy between the calculated rate of cadmium input to the oceans by rivers and the rate of cadmium sedimentation is too large to be attributed solely to an abnormality in the composition of the St Lawrence River, especially in view of a recent analysis of Amazon River water[11]. It is probable that the sedimentation rate is a more realistic estimate of oceanic throughput than river input in this case but some re-examination of both the extent of nearshore precipitation and the Cd distribution in marine sediments seems warranted. Co and Ni are the only elements for which the stream flow residence times are greater than the sedimentation rate residence times. This suggests that other forms of input are more important for these elements than the others. It is interesting that when the relative metal contents of terrigenous rock and corrected river flux are compared, Co and Ni are the only elements which are enriched in terrigenous rock. Dry atmospheric fallout may, therefore, be more important for Co and Ni than for the other elements.

Overall, our estimates of residence time are generally shorter than values given in previous compilations[15,16]. This feature is most pronounced in the cases of Fe and Mn. Labeyrie *et al.*[17] have estimated, on the basis of weapon-produced ^{55}Fe settling rates, that the residence time of Fe is about 10 yr. We are thus not alone in proposing such a short residence time for this element. Concern has often been expressed about the difficulty of rationalising residence times much shorter than oceanic mixing times with evidence for the relatively homogeneous distribution of the same elements in ocean sediments. This conflict will be reconciled if biological activity and horizontal dispersion/mixing of both particulate and dissolved metals is sufficient to ensure reasonably uniform metal concentrations in surface waters beyond the continental shelves. Settling of the inorganic and refractory biogenic components of the uniformly distributed particulate matter will then account for the relatively uniform distribution of the metals in the sediments. The residence time of Fe in the surface layer of the open ocean, calculated according to the method of Goldberg *et al.*[16] and using data from the western North Atlantic[9], is 16 yr compared with 20 yr for water. Mn, unlike most other metals, has a concentration in the surface layer which exceeds that in deep waters[9]. This suggests that its surface layer residence time is comparable with that of water. Thus it is likely that both these metals will exhibit fairly uniform distributions in open ocean surface waters despite their short oceanic residence times. The overall residence time of Fe corresponds to a settling rate of 4×10^{-6} m s^{-1} compared with 10^{-5} m s^{-1} for particles containing ^{55}Fe (ref. 17). The equivalent Stokes particle size for inorganic detrital material is about 3 μm. Nevertheless, the primary host for downward transport of Fe in the open ocean is likely to be larger, lower density, biogenic particles, such as faecal pellets, with similar settling velocities.

Metals which remain predominantly in solution are removed from the water column considerably more slowly. Their distributions in the surface layer are probably controlled by mixing and metal incorporation into the biological components that are largely regenerated in the mixed layer. The deep water dissolved metal concentrations will be maintained by the degradation and dissolution of biogenic detritus and the balancing accumulation of metals on to exposed inorganic particle surfaces in the water column and in the sediments. The residence times of the predominantly dissolved metals, therefore, probably represent the time scales in which organic scavenging, biogenic particle settling and dissolution, and final association with inorganic particulate surfaces in the deep water are occurring.

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DNA arrangement in isometric phage heads

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DNA is wound tightly into phage heads in such a way that it tends to form layers concentric with the rigid protein shell. In P22 and wild-type lambda, DNA completely fills the internal volume, with a highly uniform local packing of adjacent segments; in lambda deletion mutants containing less than a full genome, the local packing distance increases correspondingly.

DETERMINING the packing geometry of nucleic acid in virus particles presents a rather difficult experimental problem. In the heads of double-stranded DNA-containing bacteriophage, such as λ or P22, the nucleic acid is condensed 250-fold, and DNA strands are then so closely packed that conventional electron-microscopic techniques have yielded no interpretable images. Moreover, such phages are too large for application of the X-ray diffraction methods used to visualise single-stranded RNA in tobacco mosaic[1] and tomato bushy stunt viruses[2]. A large number of indirect approaches have been tried (see ref. 6), but virtually the only direct data bearing on the problem of DNA arrangement are those of Richards *et al.*[3], who managed to visualise gently disrupted bacteriophage particles in the electron microscope. They concluded that DNA

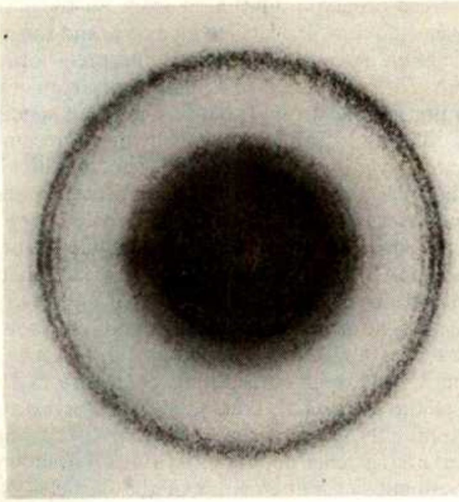


Fig. 1 X-ray diffraction from phage P22. The outer set of rings corresponds to the 25 Å diffraction discussed in the text and shown as a scan in Fig. 2. The experimental conditions are described in ref. 4.

is coiled into the phage head—either coaxially like thread on a spool or with random direction like a ball of yarn. Recent small-angle X-ray scattering studies of intermediates in the P22 head-assembly pathway led us to recognise evidence for considerable long-range order in packaged DNA[4]. We describe here an extension of that work, leading to some conclusions about the organisation of DNA in bacteriophages with isometric heads—P22, λ and T7.

Diffraction from phage

A photograph of X-ray diffraction from a solution of phage P22 is shown in Fig. 1. The series of rings at small angles can be interpreted in terms of the radial distribution of density in the particle[4]. They show that in first approximation, the P22 particle diffracts like a uniformly filled sphere. The strong outer band, corresponding to a spacing of about 25 Å in the structure, is due to DNA[4,5]. A densitometer trace of the intensity in this band is shown in Fig. 2. The diffraction is modulated by a series of ripples, having a periodicity approximately equal to the reciprocal of the head diameter. Experiments with other isometric phages (various strains of λ, T7) show this modulation to be not a mere superposition of other diffraction but rather a consequence of the geometry of DNA packing. Details of the interpretation of these observations are presented below. At wider angles, the diffraction from P22 and λ phage heads show the characteristic B-form DNA pattern, with maxima near 12, 8 and 3.4 Å (ref. 4).

Organisation of phage DNA

The diffraction from phage DNA *in situ*, just described, reveals both local and long-range order in the phage head. The presence of local order, indicated by the strong 25-Å diffraction, ha sbeen recognised for some time[5,6]; the long-range order, indicated by the rippled modulation of this diffraction, was observed more recently by Earnshaw *et al.*[4], who first examined isometric phage with X-ray cameras of sufficient resolution.

Local order: adjacent segments of the DNA molecule are packed in a locally parallel array, with a side-to-side spacing that can be determined from the position of the 25-Å diffraction band[4]. From the width of this band, we can conclude that this spacing is quite uniform throughout the phage head with an 'apparent domain size' of −125 Å (refs 4, 5, 16). Assuming hexagonal packing, as in concentrated gels of DNA *in vitro*[7], the interhelix distance is equal to 1.16δ where δ(~ 25 Å) is the spacing of the observed diffraction (Fig. 4 and Table 1, where the precise determination of δ from the pattern is described). We can also calculate the area of the local 'unit cell' (Fig. 4)—the cross-sectional area occupied by one DNA strand—this area, multiplied by the length of the P22 DNA molecules, gives the volume, *V*, taken up by the packed DNA. This calculated volume is in fact equal to the internal volume of the wild-type phage head, accurately determined from the structure of empty heads[4]. These calculations are summarised in the top line of Table 1.

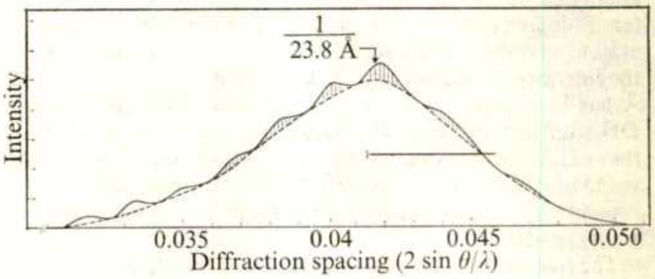


Fig. 2 Densitometer trace in the 25-Å region of a film similar to the one shown in Fig. 1. The film was scanned as described in ref. 4. The shaded peaks are those used for fitting the model in Fig. 3. The horizontal bar indicates the half-width at half-height, 0.004 Å^{-1}. The data have been corrected by subtracting a smooth background due to diffuse scatter of the X-ray beam.

Long-range order: the ripples that modulate the intensity of the 25-Å diffraction have a wavelength of about 1/540 Å, that is, approximately the reciprocal of the internal diameter of the phage head. This indicates coherent diffraction from opposite sides of a particle: locally ordered DNA segments on one side must be diffracting with a fixed phase relative to locally ordered segments on the other. A linear DNA molecule

Table 1 Measured and calculated dimensions for DNA packing in phage heads*

Phage		*d* (Å)	δ (Å)	*A* (Å^2)	*l* (Å)	*V* (Å^3)	*r* (Å)	r_0 (Å)	*n*	*f*	ref.
P22		24.2	23.8	638	14.0×10^4	8.9×10^7	278	278	4(+1)	72	8
λb221	(78)	26.2	25.8	772	12.6	9.75	285	294	5	90	9
λb2	(88)	25.1	24.7	708	14.1	10.0	288	294	5	86	9
λb515·b519	(89)	25.0	24.6	701	14.5	10.1	290	—	—	—	9
λ+	(100)	24.0	23.6	647	16.1	10.4	292	294	5	80	9
λ att^2	(105)	23.5	23.2	625	16.8	10.5	293	294	4	74	10

**d*, mean Bragg spacing of DNA diffraction band measured from films; δ, interlayer spacing, derived from *d* by applying an approximate intensity-profile correction to account for the fact that the intensity maximum will be somewhat displaced from the reciprocal of the spacing between shells; *A*, area of local unit cell of DNA segment packing (cell assumed hexagonal, so that $A = 1.16\,\delta^2$); *l*, length of DNA; *V*, volume occupied by DNA in head ($V = A.l$); *r*, minimum radius to which DNA must extend, calculated from $V = (4/3)\,\pi r^3$; r_0, radius of DNA-protein boundary, calculated from best fit in Fig. 3 by adding 12 Å to the mean radius of the outermost DNA shell; *n*, number of DNA shells used for best fit to diffraction data (Fig. 3), in P22, a protein shell has also been included; *f*, fraction of total DNA in coherently scattering shells; ref. reference for DNA molecular weight.

The number in parenthesis after the name of the lambda phage strain indicates the percentage of wild-type DNA complement present. The genotype of λatt^2 was i^{21}.b515.b519.int$_2$.xis$_4$.att^2.bio$^+$.uvrB$^+$; all other λ strains were s7 (lysis-defective). Diffraction measurements were recorded from λ in 0.05 M NaCl, 0.1 M Tris-HCl (*p*H 7.4), 0.001 M $MgCl_2$ and from P22 in 0.01 M Tris-HCl (*p*H 7.5), 0.005 M $MgCl_2$.

is not likely to form a single crystal inside a spherical phage head (for example, the recent demonstration by Lerman *et al.* of the absence of chain-folding in ethanol-precipitated DNA[19]). Coherence across the phage particle must, therefore, be imposed by the regular, isometric protein shell within which the DNA is contained. Indeed, we would expect segments of the DNA molecule, locally parallel and tightly packed against the inner surface of the phage head, to fall into layers spaced regularly inward from the protein boundary. A spherically averaged picture of the particle will thus show distinct shells of density concentric with the protein shell itself. From the degree of local order implied by the intensity profile, we expect to see three or four such shells before packing irregularities destroy the uniformity of their diameter (Fig. 4). The same mode of packing continues at smaller radii, but packing faults become increasingly frequent.

The calculation presented in Fig. 3 shows that the ripples modulating DNA diffraction from P22 can indeed arise from a shell-like density fluctuation in the particle. The calculation assumes that n-concentric DNA layers, differing in radius by increments of δ, are packed within the protein shell. This relatively tight shell[4] also contributes to the X-ray scattering since its inclusion in the calculation as a shell 24 Å from the outermost DNA layer improves the fit in Fig. 3. The radius of the outermost layer of DNA, 266 Å, has been chosen to achieve the best fit to the observed intensity profile. The radius of the boundary between DNA and protein, denoted r_0 in Fig. 4 and Table 1, can then be taken as this shell radius plus 12 Å (an estimate of the ionic radius of the DNA cylinder, see atomic coordinates in ref. 7). The parameters that best fit the intensity profile are $r_0 = 278$ Å and $n = 4$, where $\delta = 23.8$ Å has been determined directly from the mean spacing of the DNA diffraction band. This value of r_0 is, in fact, the same as the radius corresponding to V (the calculated total DNA volume). Likewise, it is in accord with the inner radius of the protein shell, determined by small-angle X-ray scattering from empty heads[4].

The trace in Fig. 2 shows that the ripples modulating the 25-Å diffraction band account for about 15% of the total intensity. Since only one of the three (100) planes in the local hexagonal lattice (marked by arrows in the insert in Fig. 4) will contribute to this sampled diffraction, we expect the ripples to account for a maximum of one-third of the integrated intensity (actually somewhat less, since the outer layers account for only about 75% of the DNA molecule). Irregularities in the lattice and layer spacing will fill in the minima between ripples, so that the observed ratio of sampled to diffuse intensity is consistent with the picture drawn in Fig. 4. Note, that the envelope of the rippled intensity follows the diffuse diffraction: the local order is essentially the same in all directions, but the packing boundary imposed by a rigid head leads to a long-range radial regularity.

Deletion mutants of phage λ

We have interpreted the diffraction from P22 phage DNA *in situ* in terms of locally parallel segments of DNA, packed tightly into the head in such a way that the polymer tends to form layers concentric with the outer protein shell. This model is summarised diagrammatically in Fig. 4. Further evidence for such a picture comes from measurements on a series of deletion mutants of bacteriophage λ. Unlike P22, which packages a constant headful of DNA, λ packages only the DNA between two distinct sites on the viral chromosome. As a result, genetic deletions result in production of phage heads containing less DNA. We have, in this way, obtained diffraction from structures with varying amounts of DNA packed into a head of constant size. The parameters of the model described above for P22 should be determined in a uniform way for the entire series of measurements on λ deletions: r_0 should be the same for all these structures, since it is in effect determined by the inner radius of the protein shell, and d should be given by the mean spacing of the DNA diffraction band, as before. Our ability to fit the observed diffraction from all the stains by a simple n-layer model, with a single value of r_0 and the measured δ for each deletion, is a strong test of this interpretation.

The results of diffraction measurements from various λ deletion strains are shown in Table 1. The mean spacing, d, of the DNA diffraction band increases with decreasing DNA content, but its half-width does not change. This indicates that the distance between adjacent segments of the DNA molecule increases, but that the uniformity of packing remains essentially the same. In other words, the degree of local order is as great in b221 as in wild-type λ, even though the total length of DNA in the head has decreased by 22% and the average spacing between adjacent segments of the molecule has increased by 9%. As shown by the calculation of V, the increase in mean intersegment spacing compensates for about two-thirds of the lost DNA, that is, V decreases by about 7% when the total DNA content decreases by 25%. This result implies that in the deletion mutants a small fraction of the DNA—presumably near the centre of the particle—is packed more loosely than the rest.

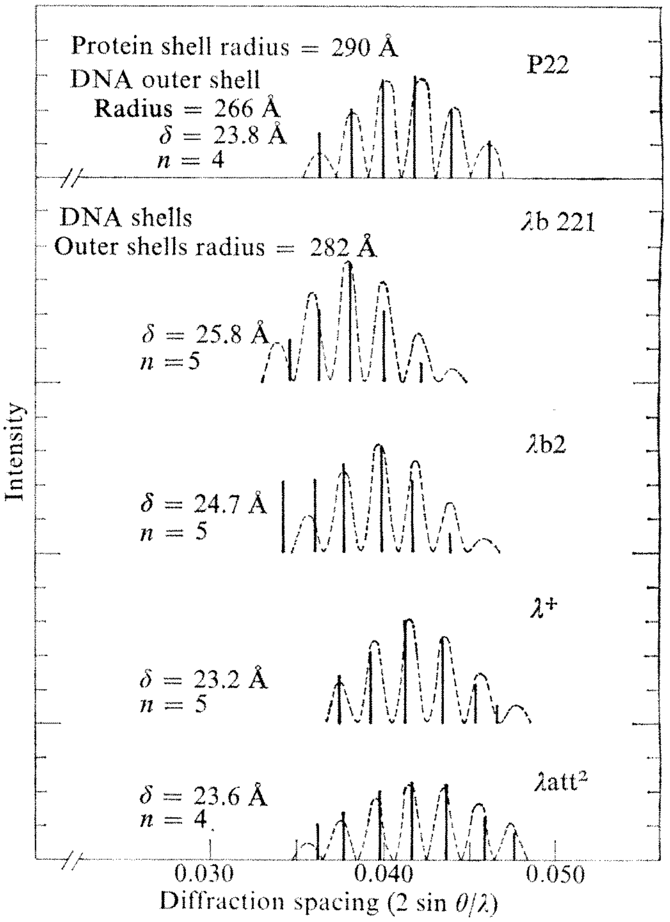


Fig. 3 Correspondence of model calculations to observed diffraction for phage 22 and various λ mutants. Spikes represent position and scaled height of intensity maxima (shaded ripples in Fig. 2); – – – – shows scattered intensity calculated for a series of concentric shells[16], with each shell weighted according to the amount of scattering material it contains. The calculated intensity equals F^2, where

$$F = \sum_{i=1}^{m} 4\pi r_i^2 \left(\frac{\sin 2\pi r_i s}{2\pi r_i s}\right)$$

and r_i is the radius of the ith shell (m in all) and $s = 2\sin\theta/\lambda$, the diffraction spacing. There are n shells of DNA spaced δÅ apart, and in the case of P22 a shell for protein has also been included. For λ mutants, where head size is constant, we have assumed a fixed radius of 282 Å for the outer DNA shell: to obtain r_0, the DNA-protein boundary, 12 Å must be added to this figure.

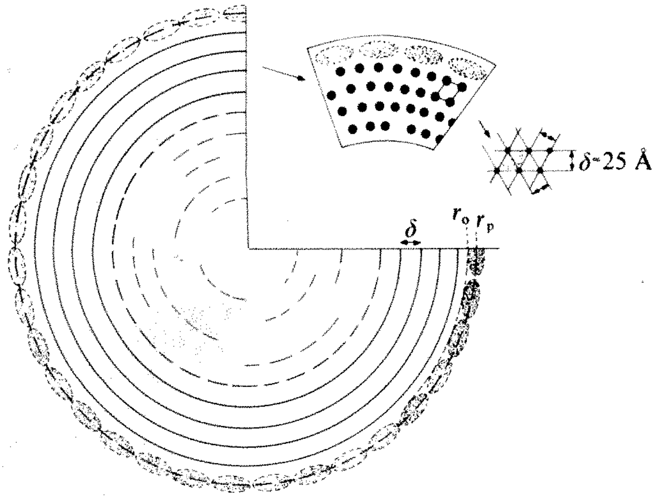


Fig. 4 Diagrammatic representation of the model used to obtain curves in Fig. 3. The protein (radius = r_p) is shown stippled; the radius of the DNA-protein boundary is r_0; the inter-shell spacing is δ. Adjacent segments of the DNA helix are parallel, with the local unit cell assumed to be hexagonal[7,19]. If this is the case, packing faults must be introduced in inner shells, as shown. For this reason, the innermost DNA is shown as partially organised shells, though it could be packed in some other manner. For phage P22 the model includes a shell at radius r_p and four inner shells with spacing δ. For the λ mutants the model includes five shells with spacing δ, the outer shell at a radius of 282 Å.

All strains studied show the same rippled modulation of the DNA diffraction described above for P22. The spacing between these ripples remains essentially constant with varying DNA content, but the positions of the maxima and minima shift, as indicated in Fig. 3. The results in Table 1 and Fig. 3 show that we can fit all observations by models with four or five coherently scattering layers, without changing r_0. That is, we can imagine an outer layer of DNA segments, packed tightly against a protein shell of fixed size, and successive layers of segments packed against the first with a spacing that increases with decreasing DNA content. Moreover, the best value of r_0 for all strains agrees well with the inner radius of urea-treated A^-petit λ, an *in vitro* analogue of empty heads (Earnshaw and Hendrix, in preparation). In contrast with P22, the protein shell itself does not appear to contribute to the 25 Å diffraction, and it has not been included in the model calculation.

The fraction of the DNA molecule that would be contained in these coherently-diffracting layers varies from about 90% for b221 to about 75% for att[2] (Table 1). In all cases, then, a large fraction of the molecule is packed in this way. There are probably several further layers, but at this distance from the constraining walls of the phage head, the segments would be sufficiently out of register that they would not contribute to ripples in the 25-Å diffraction.

The results from λ strains in Table 1 refer to phage in 0.05 M NaCl, 0.01 M Tris (*p*H 7.4), 0.001 M $MgCl_2$. Substitution of 0.01 M polyamine (spermidine or putrescine) for Mg^{2+} has no effect on the measured spacings. Moreover, diffraction from intracellular λb2.s7 accumulating in unlysed su^- cells shows the 25 Å band quite clearly. We conclude, as we did earlier for P22[4], that the arrangement of DNA in a phage head does not change when lysis alters its ionic environment. Polyamine may, however, be important for the initial packaging event[11].

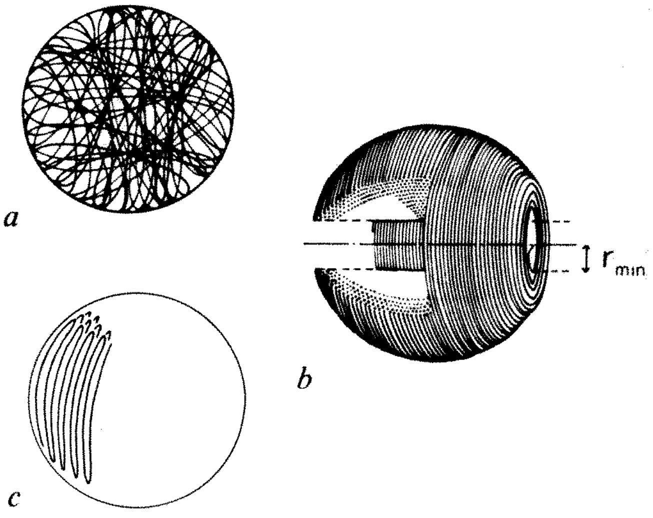
Three dimensions

What more detailed pictures of DNA packing are consistent with the picture presented above? The electron micrographs of gently-disrupted phage heads obtained by Richards *et al.*[3] show unexpanded DNA bundles, flattened into the grid: DNA strands near the periphery of these bundles always have a circumferential orientation. These micrographs suggest that DNA is not folded with sharp kinks inside the phage head, but rather, that it is wound with relatively uniform curvature. Richards *et al.*[3] proposed two limiting descriptions of DNA in phage heads based on these images: a 'ball of string' and a 'concentric spool'. In the first (Fig. 5*a*), DNA is wound roughly along a great circle path at any radius, with no particular relation of the tilt of the planes of successive great circular windings. In the second (Fig. 5*b*), the molecular windings are concentric with a single axis. Our observations seem to be inconsistent with the ball-of-string picture. The crossing of successive turns in a ball of string does not lead to parallel adjacent strands or to radially concentric layers. Such crossing also leaves gaps too large to be compatible with the tight packing of DNA in the head. Coaxial winding does not present these difficulties: segments are locally parallel and closely packed. Our data do not specify the relative orientation of these windings and the axis of the phage tail.

Certain folded structures might also be consistent with an overall organisation into shells of DNA segments layered inward from the protein, provided that the folds were appropriately positioned (Fig. 5*c*). Folds could plausibly result only from interaction of DNA with specific sites on the inner surface of protein subunits. The continuous variation of DNA spacings observed in λ deletions is difficult to reconcile with a fixed set of folding positions. Moreover, the local symmetry of the protein surface lattice implies that only radially-directed DNA can have an equivalent orientation with respect to each folding site: the uniformity of packing we have observed rules out a radial direction. Finally, we would not expect folded structures, when flattened on to an electron microscope grid, to show the images of coiled, circumferentially-directed strands seen by Richards *et al.*[3]. For these reasons, we prefer the coiled structure shown in Fig. 5*b*.

In any structure with more or less uniformly bent DNA, the stiffness of the polymer will prevent windings of very small radius, leaving a roughly cylindrical cavity in the centre of the head (Fig. 5*b*). Changes in packing of DNA in this region—estimated below to be less than 10% of the total volume of the head—might account for the slight decrease in *V* with decreasing DNA content. This could also account for the region of low density seen in the centre of λ and λ*dg* in ultra-thin sections in the electron microscope[18]. A reasonable value for r_{min}, the radius of the cylindrical cavity, is the radius of a circle of DNA for which the bending enthalpy equals ~ 7 kcal mol^{-1}—an estimate for the enthalpy of a single 180°

Fig. 5 Schematic representation of various models discussed in the text. *a* Ball-of-string. *b* Coaxial spool. *c* Chain-folded structure (with 180° folds at each end).



fold[12]. The bending enthalpy of DNA was determined by Gray and Hearst[13]: their figure of $\Delta H_{\mathrm{bend}} = 75$ kcal Å mol^{-1} rad^{-2} is applicable, strictly speaking, only to radii of curvature comparable with the solution persistence length ($\sim$ 500 Å), but its use with rather smaller radii seems adequate, since even for $r = 50$ Å the bend is only about 4° per base pair. With this value for ΔH_{bend}, we get $r_{\mathrm{min}} \sim 60$ Å. Also, using this estimate of r_{min}, integration over the total occupied volume yields $\Delta H_{\mathrm{tot}} = 4\pi(\Delta H_{\mathrm{bend}}/A))(r \ln(2r_0/r_{\mathrm{min}} - \frac{1}{2}) - r_0 + r_{\mathrm{min}}/2) \sim 500$ kcal (where A and r_0 taken here as 647 Å^2 and 294 Å, appropriate for λ, are defined in Table 1 and Fig. 4). To fold DNA into a λ head would require more than $l/2r_0$ folds (l is the DNA length; $2r_0$, the head internal diameter)—more than 2,000 kcal. The cylindrical cavity defined by r_{min} contains about 9% of the total volume. The column V in Table 1 shows that in λb221, only about 91% of the total volume inside $r_0 = 294$ Å is required for DNA packed with spacing $\delta = 25.8$ Å. In this extreme deletion, then, the central regions might actually be nearly free of DNA. In other forms, a larger fraction of the total volume is required (all of it in wild type and att[2]). In these structures, most of the DNA can be coiled into cylindrically concentric shells, but up to 10% might be folded back and forth lengthwise in the central region (a possibility suggested to J. Schellman), perhaps passing from there to the phage tail (see cross-linking experiments with λ and other phages—refs 14 and 15).

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Initiation of chick cell division by trypsin action at the cell surface

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Trypsin immobilised on polystyrene beads causes initiation of cell division which cannot be accounted for by trypsin released into the medium or into the cells. Also, initiation by soluble trypsin is inhibited by immobilised soybean trypsin inhibitor. These results demonstrate that trypsin can initiate proliferation at the cell surface.

THE site of action of peptide hormones and certain growth factors is often presumed to be at the cell surface. Indeed, binding studies and isolation of binding proteins by affinity chromatography have demonstrated that specific receptors for these molecules exist in the plasma membrane. Such receptors might be involved primarily in transport or other functions, however. For example, it has been shown that there are cell surface receptors for steroid hormones even though their site of action is clearly inside the cell[1]. There are reports that certain peptide hormones and growth promoters can act without entering cells [2–6], but most of this evidence has been obtained with Sepharose-immobilised preparations. The validity of these studies has been challenged[7–10] primarily because of the amount of soluble material released[7,8] and because such material may be 'superactive'[10]. Therefore, it is still questionable whether peptide factors can exert their biological effects without entering cells.

Trypsin and certain other proteases initiate proliferation of cultured quiescent chick embryo fibroblasts (CEF) (refs 11–18). This system has two important advantages for studying the mechanisms involved in initiation of cell proliferation. First, the initiation occurs in serum-free medium without additional growth factors[13], and second, it is caused by purified agents with well characterised proteolytic activities. In spite of these advantages, little progress has been made towards elucidating the actual mechanisms involved. It has been reported that 5-min treatments with soluble proteases or proteases linked to Sepharose beads doubles the number of mouse 3T3 cells[19–21]. This would suggest that trypsin acts at the cell surface. But other investigators have not achieved significant initiation of 3T3 cell division with trypsin using various experimental conditions including exposures of up to several days[17, 22, 23]. Even in chick cells, whose division is readily stimulated by trypsin, the protease must be present for at least 2 h to cause a significant increase in cell number, and the fraction of cells which respond increases with length of exposure up to 24 h (refs 17, 18). Such lengthy exposure times suggest a more complex mechanism of initiation than the simple binding or cleavage of specific receptors. Indeed, it has not been possible to correlate cleavage of specific surface membrane proteins with initiation of proliferation by proteases[14–16, 24]. Moreover, both thrombin (ref. 25 and B. M. Martin and J. P. Quigley, personal communication) and trypsin[26] accumulate inside cultured cells after exposures sufficient to initiate cell division. These considerations prompted us to develop techniques to rigorously determine if trypsin could initiate proliferation of chick fibroblasts without entering the cells.

We report here that trypsin covalently attached to poly-

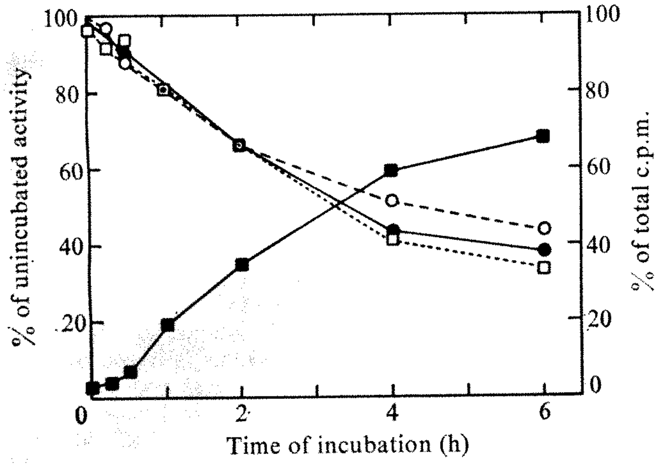

Fig. 1 Autodigestion of ^{125}I-trypsin. Correlation between TCA-precipitable radioactivity (c.p.m.), tryptic activity, and mitogenic activity. Trypsin, iodinated with Chloramine T (65,000 c.p.m. μg^{-1}), was incubated at 37 °C in PBS (*p*H 7.4). After the indicated times, aliquots were removed and assayed for acid-soluble (■) and precipitable (□) radioactivity (5% TCA), for proteolytic activity (●) as determined by hydrolysis of *p*-toluene-sulphonyl-L-arginine methyl ester[33], and for mitogenic activity (○) on quiescent CEF as described in Table 1. When compared with unlabelled trypsin, the ^{125}I-trypsin used in these experiments retained all of its mitogenic and proteolytic activity. In addition, the loss of these activities followed the loss of TCA-precipitable radioactivity. These observations show that ^{125}I-trypsin molecules are active and representative of both labelled and unlabelled molecules.

styrene beads initiates proliferation of secondary CEF. Results from various experiments have demonstrated that this initiation is not caused by trypsin released from the beads. In addition, we report that soybean trypsin inhibitor (SBTI) attached to polystyrene beads can inhibit the proliferative effect of cell-associated trypsin even if added 4 h after the cells have been exposed to initiating levels of the protease.

Immobilised trypsin preparations

In searching for a stable form of immobilised trypsin, we first tried two commercially available preparations, trypsin–Sepharose (Worthington), and trypsin–acrylamide (Sigma). Addition of optimal amounts of either preparation to quiescent CEF resulted in population doublings after 24 h. But, a cell number increase nearly as large was achieved either with medium from which the beads had been removed, or with the beads physically separated from the cells by nylon nets during the 24 h incubation. This demonstrated that the observed initiation resulted largely from released trypsin. In contrast to the above preparations, trypsin linked by carbodiimide condensation to carboxylate-modified polystyrene beads initiated division of only about half of the cells. Medium from which these beads had been removed did not initiate CEF, however, suggesting (1), that the linkage was relatively stable and (2), that the observed initiation was due to trypsin still attached to the beads. We therefore used polystyrene bead preparations for all of the following experiments.

Active component of polystyrene-immobilised trypsin

Table 1 shows results from a representative experiment which demonstrated that initiation of CEF by trypsin-beads was due to trypsin and not the polystyrene beads or a 'nonspecific' protein-bead complex. Addition of 50 μg of trypsin-beads (~ 225 beads per cell) to quiescent fibroblasts resulted in a 32% increase in cell number over control populations by 24 h. In contrast, the same amount of protein-free beads or beads with bovine serum albumin (BSA), ovalbumin, or SBTI attached did not significantly increase cell number over controls. This specificity was also observed over a wide range of bead concentrations.

Coverslip experiments

Our first attempts to determine if initiation by trypsin-beads resulted from trypsin released during incubation involved an approach where two populations of cells, one with and one without beads, shared the same medium. We cultured CEF on small glass coverslips and then added trypsin-beads (~ 225 beads per cell) to some of the cultures. Nearly all of the beads firmly adhered to the cells within 3 h. We then relocated coverslips with and without beads into new dishes. The beads remained tightly associated with the cells both during transfer of the coverslips and during the subsequent incubation. Regardless of the proportion of coverslips with and without

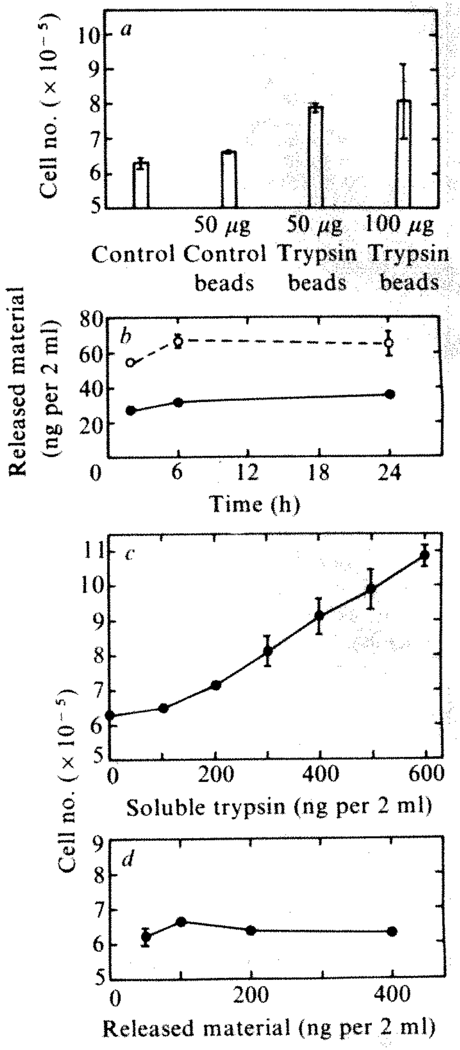

Fig. 2 Amount of material released from trypsin-beads during incubation with CEF and the mitogenic effect of different amounts of trypsin–beads, soluble trypsin, and released material. ^{125}I-trypsin (described in Fig. 1) was covalently attached to polystyrene beads as described in Table 1. 50 μg and 100 μg of the ^{125}I-trypsin-beads were added to quiescent CEF (Table 1). *a*, Cell number 24 h after bead addition. *b*, Amount of TCA precipitable material released into the medium from 50 μg (●) and 100 μg (○) of ^{125}I-trypsin-beads during the 24 h incubation. In the same experiment indicated amounts of soluble trypsin (*c*) and material released during the 2-h preincubation of ^{125}I-trypsin-beads (*d*) were added to cultures of quiescent CEF and the cell number counted 24 h later. The absence of an error bar means it was smaller than the symbol. Before using TCA precipitable radioactivity as a measure of the accumulated mitogenic activity, it was necessary to determine if material released from the beads was degraded to TCA soluble material during incubation. 0.5 μg of soluble ^{125}I-trypsin added to CEF cultures remained TCA precipitable during a 24-h incubation. Therefore, it is unlikely that material was released in an active TCA-precipitable form and then degraded.

Table 1 Effect of bead preparation on CEF cell number

Addition	Cell number ($\times 10^{-5} \pm$ s.d.)	% Increase over control
None	6.28 ± 0.30	
Beads alone	6.27 ± 0.02	−0.2
BSA-beads	6.48 ± 0.28	3.2
Ovalbumin-beads	6.56 ± 0.06	4.5
SBTI-beads	6.51 ± 0.24	3.7
Trypsin-beads	8.32 ± 0.16	32.5

Secondary chick embryo fibroblasts, obtained as described by Rein and Rubin[31], were seeded at 5.5×10^5 cells per 35-mm dish in 2 ml of medium 199 containing 2.0% Tryptose phosphate broth and 2% chicken serum. After 4 h the medium was changed to medium containing 0.02% chicken serum. After 48 h, 50 μg of beads alone or beads with the indicated proteins attached were added to the quiescent cultures and cell number was determined 24 h later. The data in all tables and figures are means of duplicate determinations. Trypsin-beads were prepared as follows: 500 μg of trypsin (three-times recrystallised, Worthington) was mixed with about 5 mg of carboxylate-modified polystyrene beads (0.926 μm diameter, Dow Diagnostics) in 0.1-M sodium acetate, *p*H 6.0, and linked by addition of water soluble carbodiimide (1-cyclohexyl-3(2-morpholinoethyl)-carbodiimide metho-*p*-toluene sulphonate, Sigma) to 0.1 M (ref. 32). After 4 h of stirring at 4 °C the beads were *a*, washed five times by centrifugation (10,000*g* for 10 min) through the sodium acetate buffer; *b*, pre-incubated at 37 °C for 2 h in conditioned medium (taken from CEF cultures 40 h after changing to 0.02% serum medium), and *c*, washed and resuspended in 2.5 ml of phosphate buffered saline (PBS). 50 μg of these beads (1.2×10^8 beads) had approximately 2.0 μg of trypsin attached. All other protein-beads were prepared as described for trypsin. During the above 2 h pre-incubation there was considerable release of trypsin-derived material. Therefore, we always included this pre-incubation to minimise the amount of material released when beads were added to CEF.

beads in any single dish, we found a 50% increase in cell number after 36 h on coverslips previously overlaid with trypsin-beads, but no increase in cell number on coverslips without them. Moreover, the amount of initiation observed in bead-contacted cells was not decreased by doubling the volume of the medium. Because initiation of CEF is very sensitive to changes in soluble trypsin concentration (see Fig. 2*c*), these experiments strongly suggested that released trypsin was not responsible for the initiation observed with trypsin-beads.

Measurement of released material and assessment of its activity

To quantitate the release of trypsin from beads during incubation, we attached ^{125}I-trypsin to polystyrene beads as described in Fig. 2. When these trypsin-beads were added to CEF, a small amount of material was released into the medium. Therefore, it was important to determine if the released material was intact trypsin and if it retained mitogenic activity. When fractionated on Sephadex G-75 columns, only a small portion of the released material was intact trypsin (23,000 molecular weight). The majority was peptide fragments with molecular weights of 12,000 or less. To determine if this material was active necessitated a very sensitive measure of mitogenic activity. Fortunately, we observed that during autodigestion of ^{125}I-trypsin the loss of mitogenic activity (as well as proteolytic activity) closely followed the decrease in trichloroacetic acid (TCA)-precipitable radioactivity (Fig. 1). Thus, simply measuring the TCA-precipitable radioactivity released from ^{125}I-trypsin beads during incubation with CEF indicated the maximum amount of released mitogenic activity.

Figure 2 shows representative experiments which demonstrated quantitatively that the amount of material released from trypsin-beads could not account for the observed initiation of cell division. The addition of 50 μg of trypsin–beads to quiescent CEF cultures resulted in a 25% increase in cell number over controls by 24 h (Fig. 2*a*). During this 24 h incubation 35 ng of TCA precipitable material was released into the medium (Fig. 2*b*). It is clear that this 35 ng of released material could not account for the initiation, since addition of 100 ng of soluble trypsin had virtually no effect on cell number by 24 h (Fig. 2*c*). In fact, to cause a 25% increase in cell number would require approximately 280 ng of soluble trypsin (Fig. 2*c*). Thus, even if all of the released TCA-precipitable material were active, eight times more would be required to account for the initiation observed with 50 μg of trypsin-beads. Addition of 100 μg of trypsin-beads brought about no additional increase in cell number even though twice as much material was released into the medium (Fig. 2*a*, *b*). It is noteworthy that adding up to 400 ng of released material had no effect on cell number within 24 h (Fig. 2*d*). Thus, even at high concentrations released material was not able to initiate CEF.

Can trypsin be released from beads directly into CEF?

The above experiments ruled out the possibility that release of trypsin into the medium caused the observed initiation. Another possibility, however, is that active trypsin could be released directly into the cells without appearing in the medium. We attempted to examine this possibility by incubating CEF with ^{125}I-trypsin-beads, separating the beads and cells, and determining if any radioactivity remained with the cells. In these experiments, however, we were unable to separate most of the polystyrene beads from the surface of intact cells by treatments with trypsin, hyaluronidase, neuriminidase, collagenase, or low concentrations of detergents. Therefore, we rinsed the cells, dissolved them in sodium dodecyl sulphate (SDS), collected the beads on Millipore filters, and measured the amount of radioactivity on the filters and in the filtrate (Table 2). As shown, soluble trypsin passed through the filters while trypsin attached to the beads was retained. SDS treatment alone caused the release of an average of 2.6% of the bound radioactivity, but no additional soluble radioactive material was observed when the beads were incubated with the cells for periods up to 24 h. These experiments ruled out significant direct uptake of trypsin from the beads by CEF.

Table 2 Recovery of ^{125}I-Trypsin attached to beads

Preparation	Incubation conditions	Soluble radioactivity (c.p.m.)	Bead-attached radioactivity (c.p.m.)	% Recovered on beads
^{125}I-Trypsin-beads (preparation A)	SDS treatment alone	1,035	26,017	96.2
	3 h with CEF	688 ± 61	$19,375 \pm 1,770$	96.6
	6 h with CEF	606 ± 13	$21,099 \pm 3,958$	97.2
	24 h with CEF	209 ± 4	$16,118 \pm 409$	98.7
^{125}I-Trypsin-beads (preparation B)	SDS treatment alone	153 ± 16	$10,895 \pm 1,395$	98.6
	24 h with CEF	151 ± 17	$9,596 \pm 375$	98.5
Soluble ^{125}I-trypsin	SDS treatment with CEF plus control beads	$12,731 \pm 2,023$	527 ± 55	4.0

^{125}I-Trypsin-beads prepared as described in Fig. 2 were added to quiescent CEF (see Table 1). After the stated incubation periods, medium and non-adherent beads were removed, the dishes were rinsed twice with PBS, and the monolayers were dissolved in 1 ml of PBS containing 2% SDS. The beads were then collected on Millipore filters (0.45 μm), the dishes and filters washed twice with PBS, and the filters and soluble material (including washes) counted. As controls, trypsin-beads from each preparation were added to dishes treated with the SDS solution and filtered. In addition, we added soluble ^{125}I-trypsin and ovalbumin-beads to CEF monolayers, immediately dissolved these with SDS, and filtered them. Since soluble trypsin was not retained on the filters in these conditions, it is unlikely that material released from beads during incubation became reassociated with beads or cellular material and was retained on the filters.

Visualisation of bead–cell interaction

When CEF are treated with soluble trypsin or thrombin they undergo morphological changes in addition to their increase in cell number. Such cells appear more elongated and slightly retracted with fewer cell–cell contacts and decreased cytoplasmic spreading[16]. Treatment with soluble trypsin also has been reported to cause the release of a protein of 250,000 molecular weight (the 'LETS' or '250K' protein) from the surface of these cells[14-16, 27]. Figure 3*a* and *b* shows CEF 24 h after addition of 50 µg of trypsin-beads and control phosphate buffered saline (PBS), respectively. Notably, the trypsin-bead-treated cells had altered morphologies resembling that reported for CEF treated with soluble trypsin. In addition, analysis of medium from cells treated with trypsin-beads revealed that a protein of approximately 250,000 molecular weight was released from the cells into the medium. These results indicated that trypsin-beads had a proteolytic effect on the surface of CEF similar to that of soluble trypsin.

Some of the trypsin-beads were endocytosed by CEF during incubation. Figure 3*c* and *d* shows trypsin-beads in contact with and inside of cells. Based on stereometric reconstruction from cross sections of 150 cells[28], we estimated that less than 4 of approximately 225 beads added per cell were endocytosed by 9 h, a time corresponding to the peak of DNA synthesis[11]. It is important to emphasise that of 100 internalised beads observed in cross sections, all appeared to be surrounded by intact membranes (see Fig. 3*d*). Therefore, even if these few internalised trypsin beads were involved in initiation of cell division, they acted on the plasma membrane and did not interact directly with the cytoplasm. Furthermore, the process of bead endocytosis itself did not trigger cell division because ovalbumin-beads, which did not stimulate DNA synthesis or cell division, were also taken up by CEF.

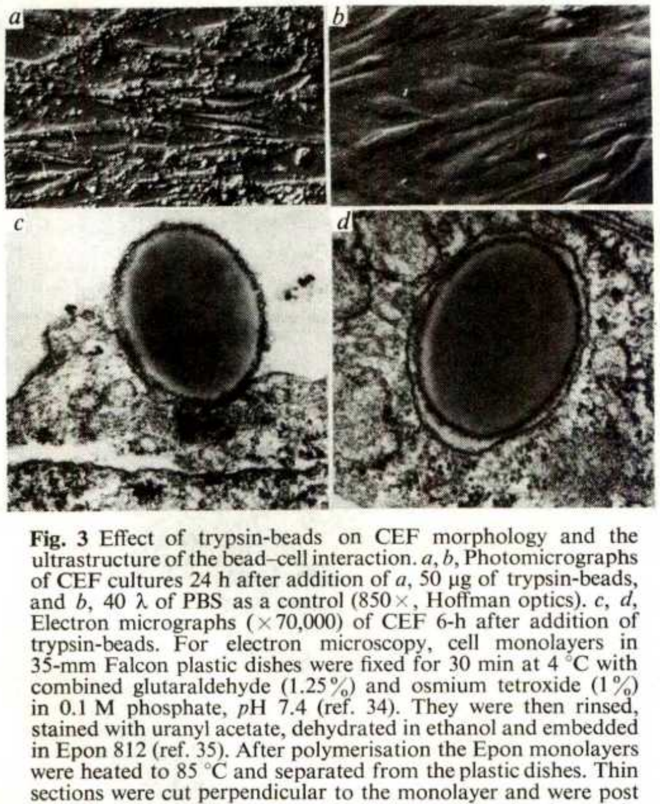

Fig. 3 Effect of trypsin-beads on CEF morphology and the ultrastructure of the bead–cell interaction. *a*, *b*, Photomicrographs of CEF cultures 24 h after addition of *a*, 50 µg of trypsin-beads, and *b*, 40 λ of PBS as a control (850×, Hoffman optics). *c*, *d*, Electron micrographs (×70,000) of CEF 6-h after addition of trypsin-beads. For electron microscopy, cell monolayers in 35-mm Falcon plastic dishes were fixed for 30 min at 4 °C with combined glutaraldehyde (1.25%) and osmium tetroxide (1%) in 0.1 M phosphate, *p*H 7.4 (ref. 34). They were then rinsed, stained with uranyl acetate, dehydrated in ethanol and embedded in Epon 812 (ref. 35). After polymerisation the Epon monolayers were heated to 85 °C and separated from the plastic dishes. Thin sections were cut perpendicular to the monolayer and were post stained with uranyl acetate and lead citrate. When beads were in contact with the surface of CEF we often observed thickened filamentous material beneath the plasma membrane (note Fig. 3*c*). But, because some beads were observed inside the cells (Fig. 3*d*) such thickenings may be involved in binding or endocytosis and not be related to initiation. That trypsin-beads were endocytosed by CEF was demonstrated by serial sectioning and by using tannic acid–glutaraldehyde as a primary fixative to show that the membranes surrounding the beads were not accessible from outside the cells[36].

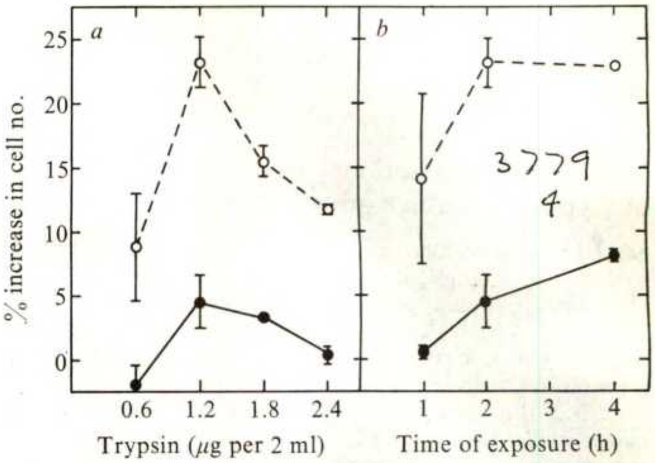

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Fig. 4 Inhibition by SBTI-beads of cell division initiated by soluble trypsin. Indicated amounts of soluble trypsin were added to quiescent secondary CEF (as described in Table 1). After the indicated periods medium containing trypsin was removed and replaced with conditioned medium taken from unstimulated parallel cultures. 120 µg of SBTI–beads were added to half of the cultures. Cell number was determined 24 h after the beginning of trypsin exposure. The absence of an error bar means it was smaller than the symbol. *a*, Effect of 2-h exposure to stated trypsin concentrations. *b*, Effect of stated exposure times to 1.2 µg of soluble trypsin per 2 ml of medium. Conditioned medium alone (○); conditioned medium plus SBTI-beads (●). Experiments with ^{125}I-SBTI–beads demonstrated that ~95% of the SBTI remained attached to the beads during incubation with CEF with or without trypsin.

Initiation by soluble trypsin can be inhibited at the cell surface

Assuming that soluble trypsin acts in the same manner as immobilised trypsin and initiates proliferation by action at the cell surface, then it should be possible to inhibit such initiation with a trypsin inhibitor bound to polystyrene beads. Indeed, in early experiments we found that addition of 120 µg of SBTI–beads to CEF inhibited 95% of the cell division initiated by 0.5 µg of soluble trypsin. But, measurements of soluble trypsin in the medium revealed that part of the inhibition could be attributed simply to a decrease in the concentration of soluble trypsin.

To bypass the problem of interpreting changes in the concentration of soluble trypsin, experiments were performed where only cell-associated trypsin was exposed to the SBTI-beads (Fig. 4). In these experiments we incubated CEF with trypsin for periods up to 4 h, and then removed the medium (containing trypsin) and replaced it with conditioned medium alone or conditioned medium plus SBTI-beads. Figure 4*a* shows the effect of 2-h incubations of CEF with various concentrations of trypsin. The highest level of initiation was a 23% increase over control cells with 1.2 µg of added trypsin (0.6 µg ml^{-1}). Addition of 120 µg of SBTI-beads (≈ 680 beads per cell) with fresh conditioned medium after the 2-h incubation with trypsin inhibited this stimulation by more than 80%. Even after a 4-h exposure to trypsin at this concentration, approximately two-thirds of the initiation could be inhibited by SBTI-beads (Fig. 4*b*). The same number of SBTI-beads only slightly inhibited serum stimulation of CEF. Thus, the inhibition was specific for initiation by soluble trypsin at the cell surface. Moreover, addition of ovalbumin beads did not inhibit initiation by soluble trypsin. These results do not exclude the possibility that soluble proteases which accumulate inside cells during long exposures might have a role in initiation of cell division. It is, however, difficult to explain how SBTI-beads inhibited initiation after 4-h trypsin exposures if the primary site of trypin action during this period were not at the cell surface.

Transmembrane action of trypsin and other growth factors

Several growth promoters have been reported to act at the cell surface. For example, there are reports that concanavalin A, phytohaemagglutinin, and pokeweed mitogen initiate DNA synthesis in lymphocytes while immobilised on Sepharose beads or plastic surfaces[5,6]. There is, however, a report that preparations of these mitogens which are active when soluble lose all of their mitogenic activity when immobilised by techniques similar to those used in the earlier reports[9]. Thus, it is uncertain whether these mitogens act at the cell surface or not.

Two other peptide factors immobilised on Sepharose beads have also been reported to act at the cell surface. (1) There are reports that insulin-Sepharose is biologically active[2,3], but recent experiments have demonstrated that all or most of its biological activity can be attributed to released soluble insulin[7,8]. (2) Nerve growth factor–Sepharose has been reported to stimulate neurite extension from sympathetic ganglia[4]. In these experiments, controls were included to measure release from the beads. These controls, however, were indirect and involved diffusion through plasma clots. With such controls it is not possible to exclude release from beads directly into the cells. In addition, the validity of experiments with Sepharose-immobilised peptide factors has been challenged on the basis that incubation of these preparations with proteins can cause release of 'superactive' factors (often with altered specificity)[10]. These problems with Sepharose preparations and the necessity for definitive controls led us to seek a more suitable method for immobilisation and to directly quantitate material released into the medium and into cells.

The experiments we report with trypsin attached to polystyrene beads demonstrate that trypsin action at the cell membrane is sufficient to initiate proliferation of CEF. In addition, the inhibitory effect of SBTI-beads on initiation by soluble trypsin indicates that soluble trypsin can also act at the cell surface to initiate proliferation. It remains to be seen how the mechanism of initiation by trypsin relates to that of other growth factors. It has been suggested that proteases may bind to or cleave receptors for more specific growth factors, and thus mimic their actions[29,30]. If so, the ability of trypsin to initiate CEF by action at the cell surface implies that these growth factors also act at the cell surface.

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letters to nature

The binary pulsar observed at four radio frequencies

PSR1913+16 is still the only known pulsar which is a member of a binary stellar system, as such, it offers the possibility of several new investigations into both the nature of pulsars and general relativity. Unfortunately, the pulsar is very weak and previous observations[1,2] have only been made with the 1000 ft telescope at Arecibo at a frequency of 430 MHz. We report here observations at four radio frequencies which show that this pulsar has properties which are similar to those of the other pulsars.

The 250 ft MK IA telescope at Jodrell Bank was used for the observations, in 1975 and 1976, at frequencies of 327, 408, 610 and 1,420 MHz. Dispersion-removing receivers were used at all frequencies providing total bandwidths of 1, 1, 4 and 4 MHz respectively, each of which was divided into 32 equal contiguous sub-bands. The time resolution is then limited either by dispersion within these sub-bands or by the integration bin size used. The integrations were carried out by forming on-line sub-integrations of 5 or 10 min in length, using the orbital ephemeris published by Taylor *et al.*[2]. These sub-integrations were added together later for total periods of between 8 and 12 h, amounting to between 1 and 1½ orbital periods of the binary system. This was achieved using the data reduction technique described by Lyne *et al.*[3], after slight modifications to compensate for the motion of the pulsar in the binary system.

The pulsar integrated profile obtained at each of the four frequencies is shown in Fig. 1 in which the whole of the 59 ms period of the pulsar is represented. The observations were made on different occasions, and have been arbitrarily aligned in phase. In all cases, the profile seems to have two main components whose separation does not vary significantly with frequency. The profile shape is characteristic of many other pulsars, although the profile covers a larger fraction of the pulsar period (~20%) than for the majority of pulsars. An unusual feature is that there does not seem to be any significant broadening of the pulses at low frequencies by scattering in the interstellar medium. Any broadening present amounts to only a few ms at 327 MHz which is rather small for a pulsar having a dispersion measure of 167 cm^{-1} pc (ref. 4).

The spectrum of the pulsar derived from these observations is of some interest and is shown in Fig. 2. There is a certain amount of variability in intensity over timescales of months in some pulsars[5], if such a variation is occurring in PSR1913+16 the measurements in the diagram, being spaced by some months, may have effective errors somewhat greater than the errors of measurement indicated. The spectral index in the range 327 MHz to 1420 MHz is measured as -1.5 ± 0.3 which is typical of many of the other pulsars[6]. Further observations, more closely spaced in time will be required to confirm this value.

Observations are continuing with the aim of examining the evolution of the orbital parameters of the binary system and of studying any associated changes in the intensity and polarisation of the radio emission. In particular, if precision of the pulsar occurs it may be possible to view the pulsar from a different rotational latitude, which is something that is not possible for any other known pulsar.

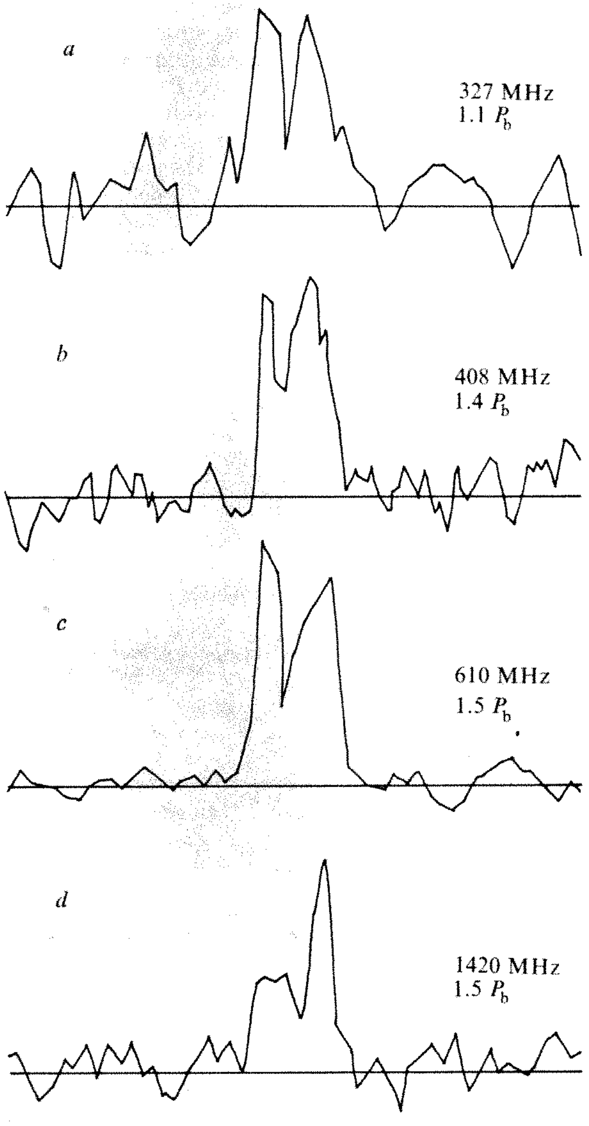

Fig. 1 The PSR1913+16 pulse profile at four radio frequencies. *a*, 327 MHz, February 1976; *b*, 408 MHz, May 1976; *c*, 610 MHz, April 1975; *d* 1420 MHz, June 1975. In each case, the whole of the 59-ms pulsar period is shown. The integration time in terms of the binary orbital period $P_b \approx 7.7$ h is also indicated.

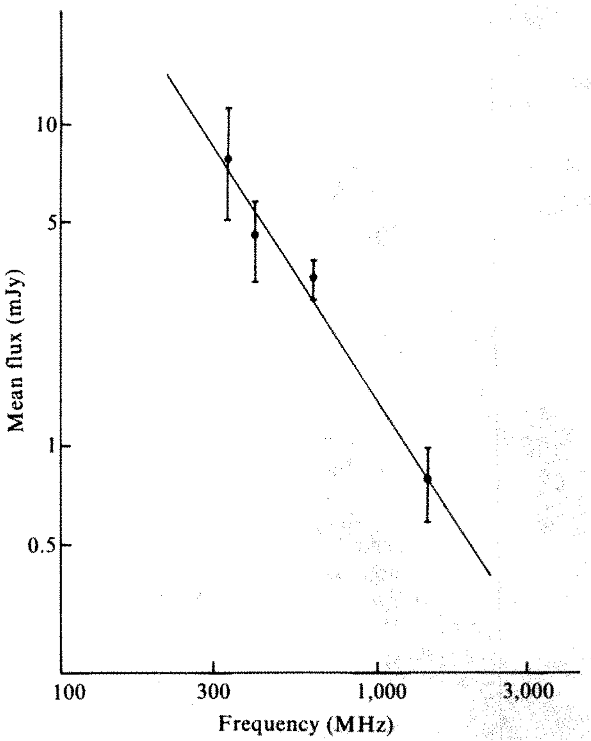

Fig. 2 The radio spectrum of PSR1913+16.

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X-ray bursts from dense clouds

X-RAY heating of matter accreting on to a compact object can create unstable flows leading to irregular X-ray emission[1–4]. Previous discussions of this instability have concentrated on the case of spherically symmetrical infall onto a stationary object. The rise-time of X-ray variations is then related to the free-fall timescale from the choking or accretion radius, whichever is relevant. Phenomena due to the presence of a magnetosphere[4] may, however, reduce this estimate. It is not clear that bursts with duty cycles as short as 10^{-3} can arise from the heating instability. Such bursts are observed, and here I investigate the effect of the motion of a compact object through a relatively dense medium. The passage of the compact object through a denser shell, which builds up outside the X ray-heated gas, produces a short burst of X rays. This creates yet another hot region leading to the repetition of bursts on a crossing time. Such behaviour may be relevant to the weak bursts possibly associated with the Cómetary Globule NGC 5367 (ref. 5), and the much shorter timescale luminous X-ray bursts[6], some of which are associated with globular clusters.

Consider first what happens when a neutron star or white dwarf, with velocity v km s^{-1}, accretes from a dense cloud such as NGC 5367. Much of the material is probably in the form of molecular hydrogen of density $n_4 \sim 1$ where $n \sim 10^4 n_4$ cm^{-3} at a temperature $T_2 < 1$ where $T = 10^2\ T_2$ K (ref. 7). X rays with energies $\varepsilon \gtrsim 2$ keV pass through such a cloud with little attenuation since it is only a few parsecs in size. The accretion radius

$$R_{\mathrm{A}} \simeq \frac{10^{16}}{(T_2+v^2)}\ \mathrm{cm}$$

yields luminosities $L \sim 10^{39}\, n_4(T_2+v^2)^{-3/2}$ erg s^{-1} in the case of a neutron star, and $L \sim 5\times 10^{37}\, n_4(T_2+v^2)^{-3/2}$ erg s^{-1} in the case of a $1 M_\odot$ white dwarf. The short-wavelength radiation, $\varepsilon > 13.6$ eV from such luminous sources would heat the accretion flow above 10^4 K, resulting in instabilities on a free-fall timescale, $t_{\mathrm{ff}} \sim 10^{11}\ R_{16}{}^{3/2}$ s, where R_{16} is the choking radius in units of 10^{16} cm.

Short timescales occur if the compact object has a substantial velocity, $v \gtrsim 100$ km s^{-1}. Many lone neutron stars may perhaps be expected to travel at such velocities, and I now concentrate on this possibility. An accretion radius $R_{\mathrm{A}} \simeq 10^{11}\ v_{300}{}^{-2}$ cm yields a luminosity $L \sim 3\times 10^{31}\, n_4\, v_{300}{}^{-3}$ erg s^{-1} and $t_{\mathrm{ff}} \sim 3\times 10^3\ R_{11}{}^{3/2}$ s. The precise form of the X-ray spectrum is unknown, but the blackbody temperature of low-luminosity neutron stars is $\sim 10^5$–10^6 K, and thus much of the radiation may emerge at energies $\varepsilon < 1$ keV. The absorption of a number, N_{ph}, of soft X rays ionises the surrounding region out to a radius R_{i} such that $N_{\mathrm{ph}} \simeq \frac{4}{3}\pi R_{\mathrm{i}}{}^3\, n$, provided that the optical depth through R_{i}, $n\sigma R_{\mathrm{i}}$, exceeds unity. The optical depth criterion demands that $R_{\mathrm{i}} \gtrsim 2\times 10^{13}\ n_4{}^{-1}$ cm, given [8] the photoelectric absorption cross section, σ. This in turn requires $N_{\mathrm{ph}} \gtrsim 4\times 10^{44}\ n_4{}^{-2}$, which is adequately supplied by $\sim 10^{35}$ erg of ~ 50 eV photons. The upper limit on the timescale over which the photons can be supplied is a crossing time of R_{i}, $t_{\mathrm{cr}} \sim 10^6$–$10^7$ s, the recombination timescale being much larger, $t_{\mathrm{r}} \sim 10^9\ n_4{}^{-1}$ s. An ionised region can therefore form if a few per cent or more of the neutron star luminosity emerges in the 13.6–50-eV band, assuming $v_{300} \sim 1$.

The absorbed energy not only goes into ionisation and dissociation of hydrogen molecules and atoms, but also into heating. The resultant temperature, $T \sim 10^4$–10^5 K will cause a large pressure difference to exist between the ionised region and the surrounding molecular cloud. R_{i} thus expands supersonically at $v_{\mathrm{s}} \sim 30$ km s^{-1} into the cool exterior, shock heating and dissociating the hydrogen molecules. The expansion is not adiabatic due to the loss of energy in dissociation. The temperature drops by a factor of ~ 10 to $\sim 3{,}000$ K in this process[9], resulting in an overall 40-fold increase in density behind the shock. The material then cools to ~ 100 K in $\sim 10^5$ s provided that the ionised fraction in the cooling gas exceeds 10% (ref. 10). The overall density enhancement in what is then essentially an isothermal shock may thus amount to a factor of $\sim 10^3$, residing in a shell of thickness $d \sim 10^{-3}\Delta R_{\mathrm{i}}$, where ΔR_{i} is the amount by which R_{i} has increased. The ionised zone approximately resembles a stand-off cone lying with its axis along the direction of motion of the neutron star.

Assume that a transient increase in luminosity, perhaps due to a density enhancement, causes a larger, roughly spherical, ionised region to form. Significant deceleration of R_{i} will only occur when an equal mass has been swept up in the shell, that is $\Delta R_{\mathrm{i}}/R_{\mathrm{i}} \sim 0.3$. When the compact object reaches the shell, $\Delta R_{\mathrm{i}}/R_{\mathrm{i}} \sim v_{\mathrm{s}}/v_{300} \sim 0.1$, and thus $d \sim 10^{-4}\ R_{\mathrm{i}}$. The density enhancement in the shell causes L to rise to $\sim 3\times 10^{34}\, v_{300}{}^{-3}$ erg s^{-1} on t_{ff}, subsequently dropping on a similar timescale after d has been crossed. The total burst energy is $\sim 10^{38}$–10^{39} erg, only a few per cent of which need be in photons creating a further ionised region beyond the shell. The X-ray bursts then repeat on intervals of $t_{\mathrm{cr}} \sim 10^7$ s. The relatively short cooling time of the dense shell prevents it from being evaporated by the low steady X-ray flux.

The observed bursts, which may have come from NGC5367, were separated by ~ 200 d and each lasted for several hours[5]. The observed 2–10-keV luminosity was $\sim 10^{34}$ erg s^{-1} during each burst. The above discussion suggests that a lone, fast moving, neutron star may be responsible. The probability that such a neutron star intersects a globule of volume ~ 10 pc^3 may be as high as 0.03, if there are as many as 10^9 suitable neutron stars within a scale height of ~ 500 pc about the galactic disk. The discussion is perhaps more relevant in indicating the possibilities for detecting burst like phenomena from molecular clouds optically thin to X rays within the plane of the Galaxy. The high galactic latitude ($b \sim 20°$) of the NGC 5367 events probably aided in their detection. Slower moving neutron stars, $v \sim 50$ km s^{-1}, may lead to events of longer duration and higher luminosities, possibly resembling Aquila X-1 and 3U1630−47 (refs 11, 12). White dwarfs may also give rise to weaker burst phenomena, as approximately half the accretion luminosity emerges in the ultraviolet[13], thereby increasing their potential to create ionised zones.

A slow moving neutron star, $v \sim 10$ km s^{-1}, moving through a region of density $n \sim 10^8$ cm^{-3} can give rise to very high luminosities, $L \gtrsim 10^{38}$ erg s^{-1}. Compton heating of the accreting gas, as well as radiation pressure effects, may choke the flow at relatively small radii, $R_{\mathrm{c}} \sim 10^{11}$ cm $\ll R_{\mathrm{A}}$ (ref. 4). Accretion may still proceed beyond R_{c}, and a dense, convectively unstable shell may result. Luminous X-ray bursts repeating on a timescale $t_{\mathrm{cr}} \sim 10^4$–$10^5$ s, caused by the passage of the neutron star through the dense shell enveloping the hot bubble produced from the previous burst, can occur if $t_{\mathrm{cr}} < t_{\mathrm{s}}$, the timescale on which the shell dissipates. The actual choking process is, as discussed in ref. 4, likely to be very complex and no detailed estimate is attempted here. Magnetospheric effects, for example, can modify the structure of the burst. Nevertheless, such bursts may in principle occur, and a suitably dense region may occur in the outer envelope, $R \sim 10^{14}$ cm of a red giant star undergoing mass loss. An accretion disk may form if the accreting material has sufficient angular momentum, as might be the case if the neutron star orbits the giant. The presence of a disk would stop bursts from occurring, but, as it depends on $R_{\mathrm{A}}{}^4$, however, a disk need not occur. Bursts such as those observed from NGC6624 (3U1820−30) (ref. 14) may be related to this process. Optical photographs[15,16] of globular cluster cores indicate the presence of luminous red giants.

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Continuous increase of Hubble modulus behind clusters of galaxies

VARIOUS authors have suggested the existence of real fluctuations to Hubble's law (proportionality of redshift to distance) as a function of the position of sources on the sky map. Karoji and Nottale[1] have examined the possibility of a variation of the Hubble constant H associated with the passage of incoming light through rich clusters of galaxies. We confirm here their positive answer by a more detailed and precise statistical analysis. We show that there is an effective continuous effect depending on the relative distance of the line of sight of any given source to the centre of the intervening clusters. We thus conclude that we are dealing with a real physical effect, tied to the presence of these clusters.

It has been shown[1] that the Hubble modulus

$$h_{\mathrm{m}} = \log V - 0.2\, m = \log H - 0.2\, M - 5$$

where V is radial velocity, H the Hubble constant, m and M, apparent and absolute magnitude of a galaxy, is significantly higher for galaxies located within and behind rich clusters of galaxies (denoted region B) than for intercluster objects (denoted region A). This result has been suggested on four independent samples[1–4] and confirmed by analysis of brightest cluster galaxies[5]. The method used previously—comparison of the mean values $\langle h_{\mathrm{m}} \rangle_{\mathrm{B}}$ against $\langle h_{\mathrm{m}} \rangle_{\mathrm{A}}$ through an application of Student's t test, was a rather crude one. We present here a new method of analysis of h_{m} variations as a function of a continuous variable. Of course, if each independent sample had been rich enough, it would have been advisable to construct h_{m} 'isovalues' on the sky map. Since this is not the case, we have devised an alternative method.

First, rich clusters of galaxies being defined as in ref. 1, we classify galaxies as:
(1) Cluster galaxies, if they are situated within the limits (as defined by Zwicky *et al.* in CGCG[6]) of a cluster, and if their radial velocities V are within an interval of 6 σ around the mean cluster velocity (the mean value of σ for the clusters used in this analysis is around 750 km s^{-1}), that is

$$V_{\mathrm{cl}} - 3\sigma(V) \leqslant V \leqslant V_{\mathrm{cl}} + 3\sigma(V)$$

(2) Galaxies more distant than a cluster if $V > V_{\mathrm{cl}}$ outside Zwicky's limits or $V > V_{\mathrm{cl}} + 3\sigma(V)$ inside them.

Second, we refer each galaxy to a subsample of clusters nearer than it, or to which it belongs, and calculate the ratios θ/R, where θ represents its angular distance to a cluster centre, and R the angular cluster radius. Then we define a parameter α as the minimum of θ/R values, that is, $\alpha = \min \theta/R$. Centres and radii of clusters are taken from ref. 6.

Finally, h_{m} values are plotted with respect to α for a given sample of galaxies.

We present here the results obtained on the sample of Markarian galaxies identified in ref. 6, described and listed in ref. 4 (apparent magnitudes taken from ref. 6). This sample is of course magnitude limited ($m \leqslant 15.7$), which implies the presence of a Malmquist bias (distant galaxies are brighter in the mean than nearer ones) so that the h_{m} values increase with radial velocity. So we have built an unbiased subsample by taking only the brightest Markarian galaxies, $h_{\mathrm{m}} > 0.96$. The clusters of galaxies used here are listed in ref. 3.

Figure 1 is a plot of h_{m} against α (for $\alpha \leqslant 6$). There is clearly a continuous decrease of h_{m} for $0 \leqslant \alpha \lesssim 3$. A quantitative analysis gives highly significant results.

First we have studied the distribution of h_{m} values subdivided with respect to α into bins of 10 points each (Table 1). If cluster galaxies (4 points) are excluded, the first bin yields $\langle h_{\mathrm{m}} \rangle = 1.170 \pm 0.031$ ($0.4 \leqslant \alpha \leqslant 1.7$) instead of 1.144 ± 0.029. These values might be compared with h_{m} for $\alpha \gg 1$, which are of the order of 1.05 ± 0.02 (Table 1).

A second method is to find the regression curve corresponding to h_{m} against α values. It is clear from Fig. 1 that a single line cannot account for the whole distribution. There is a strong decrease up to $\alpha \simeq 3$, and for $\alpha > 3$, the distribution is practically flat. Indeed, the equation of the least-square line for $\alpha \leqslant 3.3$ (cluster galaxies excluded) is $h_{\mathrm{m}} = 1.217 - (0.0567 \pm 0.016)\, \alpha$, the correlation coefficient being 0.48, which means that the probability that no correlation exists between h_{m} and α is only 0.001. The results of the least-square linear fit for $\alpha \leqslant 3$ $\alpha > 3$ are

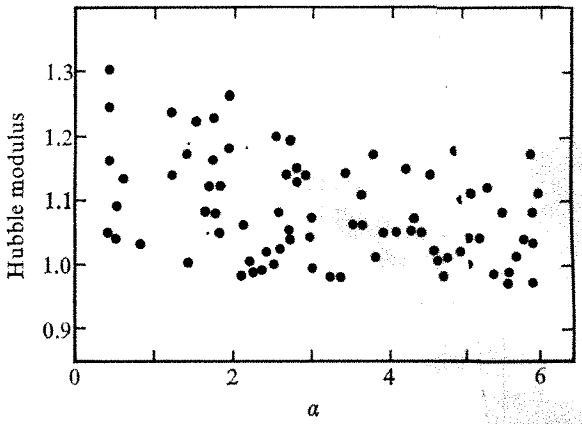

Fig. 1 Hubble modulus against normalised projected distance to nearer cluster $\alpha = \theta/R$ for unbiased sample of Markarian galaxies ($h_{\mathrm{m}} > 0.96$).

$$\alpha \leqslant 3,\ n = 38,\ h_{\mathrm{m}} = 1.207 - (0.0502 \pm 0.0183)\, \alpha,\ \rho = 0.41$$

$$\alpha > 3,\ n = 43,\ h_{\mathrm{m}} = 1.080 - (0.0048 \pm 0.0109)\, \alpha,\ \rho = 0.07$$

There is practically no correlation when $\alpha > 3$. We think that a good fit of the distribution of Fig. 1 for galaxies behind clusters is then achieved with

$$h_{\mathrm{m}} = 1.21 - (0.055 \pm 0.018)\, \alpha \text{ for } \alpha \lesssim 3, \text{ and } h_{\mathrm{m}} = 1.05 \text{ for } \alpha > 3$$

As the preceding method gives the statistical significance of only part of the distribution we have also tried to give a numerical account of it by a unique curve: Fig. 1 suggests a law of the shape $\exp(-a\alpha^2)$. We find the following best fit: $h_{\mathrm{m}} = 1.051 + (0.099 \pm 0.025) \exp(-0.17\, \alpha^2)$, significant at the 4 σ level (Fig. 2).

Finally, these results are confirmed by the analysis of the effect through the method of Karoji and Nottale[1]. The mean value of h_{m} in region B (that is, $\alpha \leqslant 2$) is 1.141 ± 0.018 ($\pm$ s.e.m.), in region B* ($\alpha \leqslant 2$, cluster galaxies excluded) 1.153 ± 0.010 and in region A, 1.057 ± 0.008. Then the differences are respectively 0.084 ± 0.017 and 0.096 ± 0.018, significant at the 4.9 σ and 5.3σ levels.

It is not the purpose of this letter to discuss possible observational explanations of this effect. This has been started in the original paper of Karoji and Nottale[1] and in refs 3 and 4. A complete analysis is now in preparation. We will only discuss two particular points.

It is possible that our method of classifying galaxies with respect to clusters has an influence on the effect. Indeed a bias

Table 1 Analysis of data from Fig. 1

(1)	α (2)	$\langle h_{\mathrm{m}} \rangle$ (3)
1—10	0.4—1.2	1.144±0.029
11—20	1.4—1.8	1.123±0.023
21—30	1.9—2.5	1.068±0.033
31—40	2.6—3.0	1.101±0.018
41—50	3.0—3.8	1.056±0.022
51—60	3.9—4.7	1.061±0.016
61—70	4.7—5.4	1.057±0.021
71—80	5.5—6.0	1.045±0.021
81—85	6.1—6.8	1.056±0.046

Data from Fig. 1 was analysed into bins of 10 points each. (1), Ranks of points in a bin; (2) α interval; (3), mean h_{m} in each bin $\pm$ s.e.m. $\sigma/\sqrt{n}$.

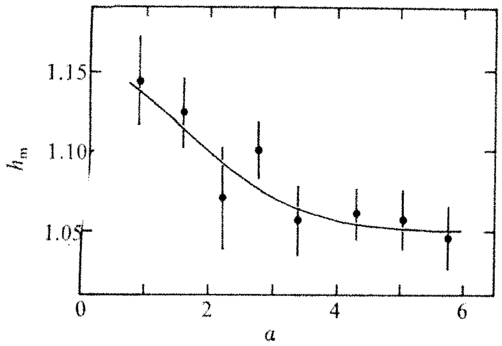

Fig. 2 Mean values of h_m against α in bins with errors bars and regression curve (see Table 1 and text). The last bin, containing only five points, is excluded.

could be present if clusters are actually greater than given by CGCG limits. So we have used another distance criterium, that is, galaxies are more distant than a cluster if $V > V_{cl} - 3\sigma(V)$. This change introduces no difference in our results, since only four galaxies are concerned by it, three slightly, and one ($h_n = 1.04$) having $\alpha = 5.1$ changed in $\alpha = 2.2$.

The second point is the possible relation with the Rubin *et al.* effect[7,8] which is now interpreted by them as a motion of the local group with respect to ScI galaxies ($3{,}500 < V < 6{,}500$). The simplest way to take it into account is to correct our data for this motion ($V_{GL} = 450$ km s^{-1} towards $\alpha = 4$ h 12 min, $\delta = 36°$). Our results are practically not affected by this correction. We obtain $\langle h_m \rangle_B{}^* - \langle h_m \rangle_A = 0.090 \pm 0.018$ ($t = 5.0$) and $\langle h_m \rangle_B - \langle h_m \rangle_A = 0.076 \pm 0.017$ ($t = 4.5$). It thus seems clear that if this solar motion exists, it is a small effect and independent of the Karoji–Nottale effect at the distances involved with our sample of Markarian galaxies ($V \sim 10{,}000$ km s^{-1}).

Remembering that this analysis was performed on a subsample not affected by a Malmquist bias, our conclusion is that these results strongly support the existence of a dependence of redshift on the position of galaxies relative to concentration of matter, that is, clusters of galaxies.

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Polysaccharides and infrared spectra of galactic sources

Observations over the infrared waveband 2–30 μm available for a number of astronomical objects are shown here to be reconcilable with the transmittance properties of polysaccharides. Using an experimentally determined transmittance spectrum for cellulose we can readily relate astronomical data in the 2–4-μm, 8–13-μm and 15–30-μm wavebands and we obtain close fits to astronomical spectra in these several bands. From this detailed spectral agreement we consider it reasonable to infer the detection of interstellar polysaccharides. The identification of this highly complex macromolecule, presumably formed by an abiogenic processing of interstellar formaldehyde, could have a profound bearing on interstellar chemistry including the evolution of prebiotic molecules.

Physical conditions within dense molecular clouds are known to favour the production of organic molecules, probably through electron-exchange reaction chains initiated by the cosmic-ray ionisation of hydrogen and helium atoms[1,2]. The majority of such molecules are fragile, however and are easily broken up by exposure to an appreciable intensity of starlight, which they will be if the parent clouds become dispersed to the normal density of the interstellar gas. Thus, since the interstellar gas undergoes alternating phases of compression and evaporation, there must be corresponding alternations of chemical complexity—molecular dissociations taking place during evaporation and association during compression. As Sagan has pointed out[3] such alternations provide a selective process for the emergence of those chemical forms that can best withstand the adverse conditions of the evaporative phases.

With the exception of H_2, H_2O and CO the most ubiquitous and very likely the most abundant molecule in the interstellar medium is H_2CO, probably formed by electron-exchange reactions[1,2]. Formaldehyde would seem an obvious example of a fragile molecule forming within dense clouds which dissociates as soon as the clouds evaporate. This will certainly be the case for individual molecules, but formaldehyde has the remarkable property of polymerising in many ways, the polymers being far more resistant to break-up than the individual molecules.

The most refractory polymers are probably the polysaccharides, which have substructures built from H_2CO units, substructures with the empirical formula $(H_2CO)_n$, where $n \geqslant 3$. The commonest polysaccharides, cellulose and starch with $n = 6$, are particularly stable because each $(H_2CO)_6$ is able to form itself into a very stable ring, with the polysaccharide then becoming a chain of hexagonal ring substructures. Cellulose can maintain its structure (in a vacuum or in an inert atmosphere of low density) probably up to a temperature of around 625–900 K. (Laboratory data for wood cellulose indicate stability up to about 620 K (ref. 4), but in low-pressure interstellar conditions and in the absence of free oxygen there could exist polysaccharides similar to cellulose which can withstand temperatures up to about 900 K.)

In accordance with the selection occasioned by the alternating phases of compression and evaporation of the interstellar gas, can we expect fragile molecules of interstellar formaldehyde to have evolved into stable polysaccharides like cellulose and starch? Without supporting evidence, this would seem a bold conclusion, to say the least. Yet with the evidence presented here an affirmative answer does indeed seem warranted.

Interstellar solid material is strongly absorbing in two infrared bands, centred at 3 μm and at 10 μm. Because crystals of water-ice absorb at about 3 μm and magnesium silicate at 10 μm, it has seemed natural to attribute the infrared properties of interstellar 'dust' to a mixture of water-ice particles[5] and magnesium-silicate grains[6,7]. Indeed both these assignments are likely to be appropriate in certain types of localised region. But the detailed correspondence of a silicate–ice model to the observed infrared spectra of a wide range of galactic sources is not good, especially if one takes account of experimentally measured absorption spectra for silicate particles[8]. The failure to obtain detailed correspondences is the more disturbing because ice and silicate grains are rather simple materials, without much scope for their properties to be changed by other substances. For such simple materials the issue should be clear-cut, yet it is not. Rather it is frequently necessary to appeal to uncertainties in the astronomical models to explain away discrepancies between observation and theory.

This was the background which made it seem worthwhile to consider whether the infrared properties of interstellar 'dust'

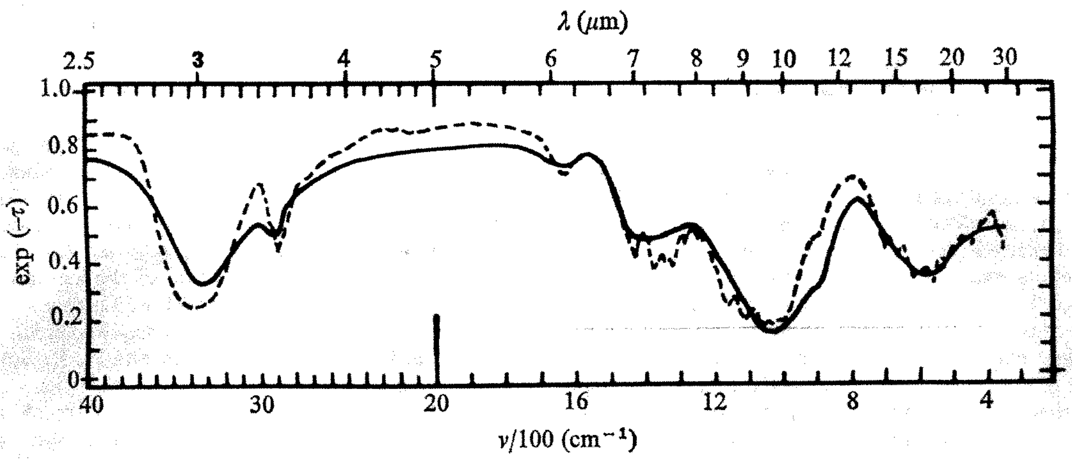


Fig. 1 Dashed curve is the transmittance data for cotton cellulose ('A cellulose 1') from ref. 9. Solid curve represents a smoother spectrum which we have estimated for an ensemble of related polysaccharides.

could be attributed to polysaccharides. Of all the polysaccharides, cellulose seems, because of its economic importance, to have been the most extensively studied, and the broken curve of Fig. 1 is reproduced from a calibrated study of the infrared spectra of cotton and its modifications[9]. Cellulose is immediately seen to have broad absorptions both at 3 μm and 10 μm, with the latter quite devoid of any unwanted spike of high transmittance, such as has been reported for silicates[8]. Transmittance values, $e^{-\tau}$, where τ is the opacity of the experimental sample, can be read off from the curve. Other polysaccharides possess similar transmittance properties, with slight wavelength displacements for the same general features (see ref. 12). The effect of such displacements for an ensemble of polysaccharides would therefore leave the general features unchanged, but must produce smoothing, as we have indicated by the solid curve of Fig. 1.

If the interstellar 'dust' has transmittance values corresponding to cotton cellulose, then the emission per unit wavelength band from an optically thin cloud of 'dust' at temperature T will be proportional to $\tau\ B_\lambda\ (T)$, where B_λ is the Planck function (This assumes the validity of the Rayleigh approximation for small particles with non-resonant absorptions. Technical issues relating to this assumption will be discussed elsewhere). Choosing the constant of proportionality to give a normalisation of 2.00 at $\lambda = 10$ μm, and taking $T = 175$ K, we obtain the dashed curve shown in Fig. 2. The solid curve is calculated from the estimated smoother spectrum for a polysaccharide ensemble (solid line in Fig. 1). Since these calculated curves give excellent fits to the observations for the Trapezium Nebula[10,11] (which are also given in Fig. 2) we consider that a *prima facie* case for the existence of interstellar polysaccharides has been established.

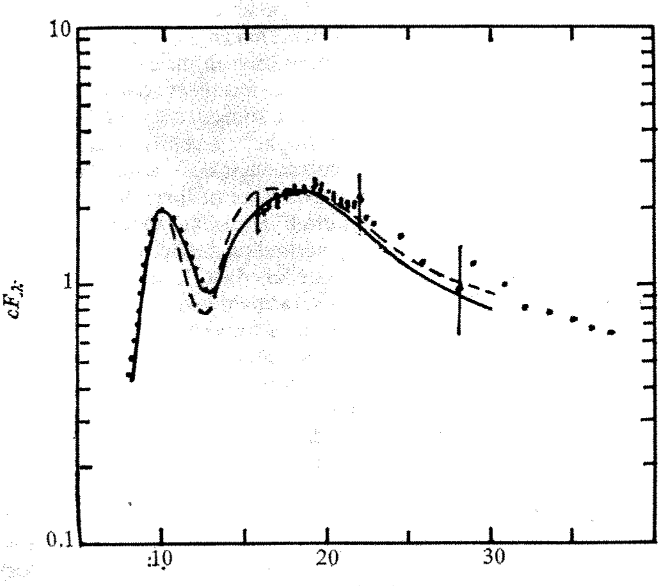


Fig. 2 Normalised flux from the Trapezium Nebula (refs 11, 12) (points) compared with emission from polysaccharide grains of temperature 175 K. Dashed curve is for cellulose (compare Fig. 1); solid curve corresponds to the polysaccharide ensemble spectrum as in Fig. 1.

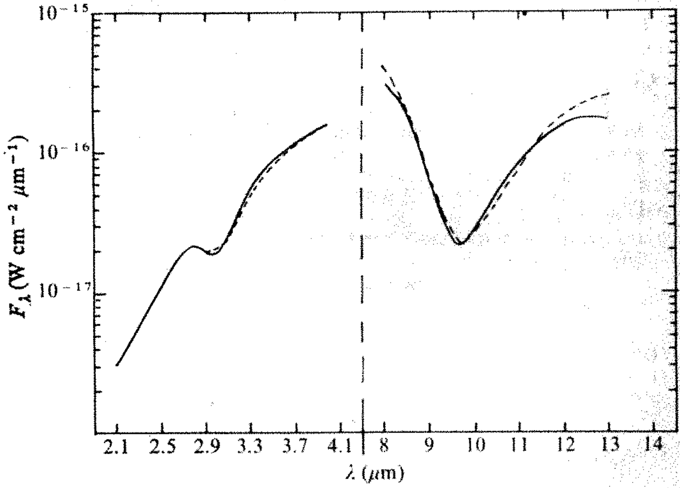


Fig. 3 Solid curve is a plot of the normalised flux against wavelength for an optically thin polysaccharide model with $T = 43$ K, $\alpha = 2.6$. Dashed curve is the observational data for the astronomical source H_2O 610+18.

It has become well known among astronomers that material with the same emissivity as that which gives rise to the Trapezium spectrum also provides a thoroughly satisfactory explanation of the observed characteristics longwards of 8 μm for a wide range of other sources (see refs 13, 14). Hence the agreement of the cellulose data (and of the smooth curve of Fig. 1 in particular) with the Trapezium Nebula already ensures that the polysaccharide data must fit the observed characteristics longwards of 8 μm for the same wide range of sources. Our concern here will therefore be directed to the shorter-wave band 2–4 μm, since it is here that a more interesting situation arises.

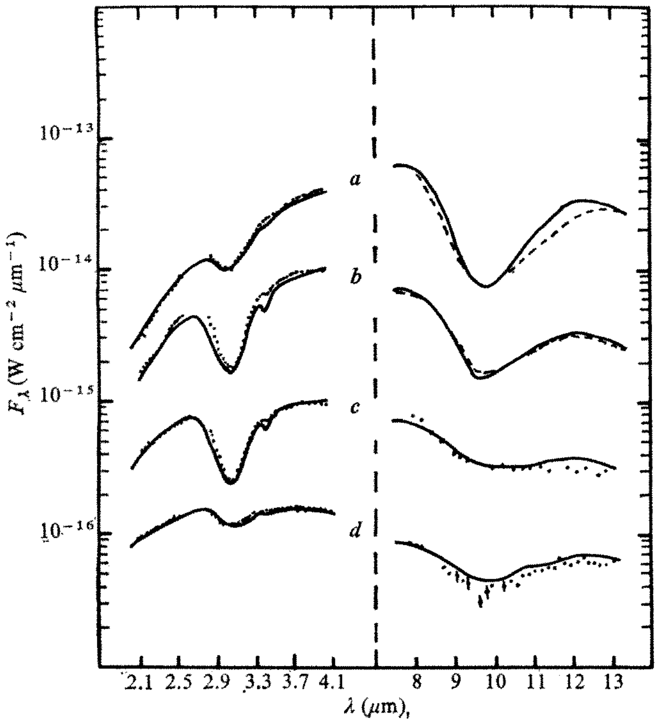
We ask two questions. First, is there a satisfactory agreement of the polysaccharide data to observation for the shorter wavelength band, 2–4 μm? And second, can the shorter wavelength band be related in its intensity to the intensity of the longer wave band?

Taking the latter question first, Fig. 3 shows the relation of observations (broken curve) for the source H_2O 610+18 to the calculated curve (solid) given by the following model:

An optically-thin emitting cloud of temperature T lies behind a foreground cloud that is cool enough for its emission to be not relevant over the wavelength range under consideration here. The relative intensity is determined in the same way as for the Trapezium Nebula, except that an extra factor $\exp(-\alpha\tau)$ must be included to take account of absorption by the cool cloud. Thus the intensity is determined by $\tau B_\lambda(T)\exp(-\alpha\tau)$. The particular values chosen to represent the source H_2O 610+18 were $T = 430$ K, $\alpha = 2.6$. The answer to the first of the above questions is therefore affirmative, since the two wavebands are properly related (see also ref. 15, which gives a similar calculation for the source OH 26.5+0.6 taken over the very wide wavelength range of 2–30 μm).

The answer to the second question is also affirmative, as can be seen from the excellent fit of observation and calculation for the four sources given in Fig. 4. The model used here for the 2–4-μm waveband was the same as that described above, except that the hot emitting cloud was taken to be optically-thick. This has the effect of simplifying the formula used in the calculation, through the omission of the factor τ, to $B_\lambda(T)\exp(-\alpha\tau)$. The values chosen for T and α are shown in the legend to Fig. 4. The details of these calculations, together with a discussion of several other sources, will be given elsewhere. In particular, the matching of the shorter and longer wavebands for these sources which involves a new feature in the model, will be discussed separately.

Fig. 4 Cases *a–d* represent observational data (points) and normalised theoretical fluxes (solid curves). *a*, Observational points for CRL2591 × 20; theoretical curve on short wave side corresponds to an optically thick polysaccharide model with $T = 650$ K, $\alpha = 0.6$. *b*, Observational points for BN × 4; theoretical curve on short wave side is for an optically thick model with $T = 650$ K, $\alpha = 1.75$. *c*, Observational points for NGC2264IR; theoretical curve on short wave side is for optically thick model with $T = 800$ K, $\alpha = 1.7$. *d*, Observational points for CRL490 ÷ 3; theoretical curve on short wave side is for optically thick model with $T = 850$ K, $\alpha = 0.25$. All the observational data are from ref. 12 and the theoretical curves are normalised to match the observations at one wavelength. The longwave calculations involve model refinements to be discussed elsewhere.



We conclude by noting that near 3 μm the sources of Fig. 4 show dips of very different depths one to another. Yet the same transmittance data was used in all cases—the difference arises from the choices of T and α. In the past, the dips at 3 μm have been attributed to water–ice, with *ad hoc* assumptions of different degrees of condensation of the ice necessary in order to explain the variability of the dips. There are no such *ad hoc* assumptions here. Nor is water–ice required at all.

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Mechanism for formaldehyde polymer formation in interstellar space

CONSIDERING the properties of galactic dust clouds in the wavelength region 8–12 μm Wickramasinghe[1] concluded that formaldehyde undergoes polymerisation in interstellar space to polyformaldehyde. Applying more stringent constraints for matching infrared spectra of galactic sources over the waveband 2.5–30 μm Hoyle and Wickramasinghe[2,3] recently argued for the identification of interstellar polysaccharides—which could be regarded as a further chemical evolution of formaldehyde polymers. A considerable fraction of all interstellar O and C may be locked up as some form of polymerised formaldehyde, making up the main component of interstellar dust. I argue here that molecular tunnelling in condensed formaldehyde is the only viable mechanism for the formation of formaldehyde polymers in interstellar conditions. Such a polymerisation process may be initiated by the action of ionising radiation.

The concentration of gaseous formaldehyde in dense molecular clouds with density $n_{\mathrm{H,H_2}} \sim 10^4$ cm^{-3} is $n_{\mathrm{H_2CO}} \sim 10^{-5}$–$10^{-6}$ cm^{-3}. Using appropriate values for the heat of polymerisation of gaseous formaldehyde with the formation of crystalline polyoxymethylene (POM) ($\Delta H = 16{,}700$ cal mol^{-1} at 1 atm) and of the corresponding decrease in the entropy ($\Delta S = 42.6$ cal mol^{-1} deg^{-1}) (ref. 4) one can easily see that at the above-mentioned $n_{\mathrm{H_2CO}}$ values interstellar POM would be thermodynamically stable below ~ 100 K. Furthermore, silicate grains of dimension $\lesssim 0.1$ μm (ejected from cool stars) which many serve as the sites of condensation and polymerisation of formaldehyde have temperatures below ~ 20 K. Thus thermodynamical considerations confirm the possibility of the formation of stable polyformaldehyde (and related polymers) in cold regions of interstellar space. In such circumstances the kinetics of polymerisation must have a decisive role.

Although direct polymerisation on the surfaces of interstellar mineral grains can be initiated by ionising radiation, the rate of propagation of polymer chains at very low temperatures could be vanishingly small[5]. Indeed, an Arrhenius-type extrapolation of our data[6–10] on the kinetics of formaldehyde polymerisation at temperatures $\sim$ 77–140 K to lower temperatures leads to the conclusion that the time for the addition of each new link to the growing polymer chain (with the activation energy $E \sim 2{,}000$–$2{,}500$ cal mol^{-1}) would be $\tau_E \sim 10^4$ yr at 20 K, $\tau_E \sim 10^{30}$ yr at 10 K and $\tau_E \sim 10^{100}$ yr at 4 K. Nevertheless, the length of the chains (υ) of formaldehyde polymerisation which is close to the radiation-chemical yield of this process $G = g\upsilon$) (g is the number of primary active centres of polymerisation produced by 100 eV of absorbed X or γ radiation; $g \sim 1$) is quite large and reaches $\upsilon \sim 10^7$ at 140 K, $\upsilon \sim 10^5$ at 77 K and $\upsilon \sim 10^3$ at 4 K (refs 6–10). At first sight the value of $\tau_E \sim 10^4$ yr would seem compatible with the possibility of an Arrhenius-type, over-barrier growth of sufficiently long POM chains in interstellar grains, because the lifetime of dust clouds limited by their gravitational collapse or by clould–cloud collisions is typically $\sim 10^5$–10^7 yr (refs 2,3,11). As will be shown below, however, an insurmountable obstacle for the over-barrier growth of polymer chains is the saturation of the radiation-chemical formation of chain propagating active centres—radicals or ions.

The steady-state concentration of radicals R_s achieved in conditions of external irradiation of molecular solids, even at very low temperatures ($\lesssim$ 4 K), corresponds to rather small saturation values: $R_s \sim 10^{-3}$–10^{-2} and does not depend on the intensity of radiation. In principle there can be two conceivable causes of such saturation:

(1) Radicals and other active centres can be formed in solids only in the vicinity of various traps of energy—for example, defects and impurities. In such a case active centres formed by radiation can remain for a very long time (practically an infinite time) localised at some imperfections. Thus R_s is simply the fraction of pre-existing imperfections in the solid and obviously does not depend on the intensity of external radiation as long as the formation of additional defects by radiation itself can be neglected.

(2) The disappearance of radicals formed by radiation is due to recombination, and moreover the rates of both formation and recombination of radicals are proportional to the intensity of radiation[13]. In such circumstances R_s would again be independent of radiation intensity.

In this latter case[2] active centres are spatially delocalised and therefore R_s is equal to the part of the time (x_{act}) spent by each molecule in an active state. As shown by numerous experiments (see, for example, ref. 11) it is precisely this mechanism which causes the saturation of the yield of radicals at 77 K. Investigations[13] of the formation and disappearance of radical pairs at much lower temperatures (down to 1.5 K) have demonstrated that the migration of radicals proceeds at least down to 35 K and most probably even to lower temperatures. Therefore R_s characterises the value of $x_{act} \sim 10^{-3}$–10^{-2} rather than the degree of imperfection of the solid. (Migration of free radicals as such is not required for the correctness of the treatment of R_s values as a measure of the time-probability rather than space-probability of finding an active centre at any site in the solid. The equality $R_s = x_{act}$ which is the premise of following estimates can be fulfilled for any other mechanism of free valency migration—for example in cases of tunnelling of H-atoms and/or electrons which is certainly feasible even at very low temperature.)

Assuming that active centres are produced by ultraviolet radiation[10] and that the density of quanta $\rho_{UV} \sim 10^{-2}$ cm^{-3}, that is their flux $j_{UV} = \rho_{UV}c \sim 3 \times 10^8$ cm^{-2} s^{-1}, we obtain the rate of absorption of ultraviolet quanta by interstellar dust molecules as 3×10^{-10} s^{-1}, so that the characteristic time between two successive ultraviolet absorptions by a molecule is $\tau_{UV} \sim 100$ yr. Meanwhile the fraction of time spent by any monomer molecule in its active state when it is able to participate in chain propagation is only $\tau_{act} = x_{act}\ \tau_{UV} \sim 0.1$–$1$ yr. When $\tau_E > \tau_{act}$, the probability of formation of a polymer chain of υ links is

$$w_v = [\tau_{act}(\tau_{act} + \tau_E)^{-1}]^{v-1}$$

This expression decreases very fast with the increasing υ and therefore for the above-mentioned value of τ_E (20 K) $\sim 10^4$ yr, given by an Arrhenius-type extrapolation, the formation of even trimers is extremely improbable.

The validity of an Arrhenius-type extrapolation of data for $T \geq 77$ K to lower temperatures is highly suspect, however. In the conditions of our experiments[5–9] when the dose rate of ^{60}Co γ radiation (which initiated polymerisation chains) was ~ 10 rad s^{-1}, the characteristic time for absorption of γ quanta was also close to $\tau \sim 100$ yr, and $\tau_{act} \sim 0.1$–1 yr. Even at temperatures as low as 4 K the length of polymerisation chains reached $\upsilon \sim 10^3$. This implies that the time for the addition of one new link to the growing polymer chain τ_0 had to be much shorter than 10^{-3} $\tau_{act} \sim 10^3$–10^4 s.

Indeed, our experiments[5–9] have shown that the above-mentioned extrapolation of the average time τ_0 for the addition of one new link to the growing polyformaldehyde chain, based on Arrhenius' law, is absolutely inadmissible below temperatures around 77 K. The increase of τ_0 with the decrease of temperature in this region sharply slows down, and reaches an asymptotic plateau value: $\tau_0 \sim 10^{-2}$ s, below ~ 10 K with no further increase at lower temperatures. Such a value of τ_0 is $\sim$ 5–6 orders of magnitude below the value of τ_0 necessary for the formation of chains with $\upsilon \sim 1{,}000$ at 4 K. This conclusion, based on direct laboratory data, has a very important bearing on the existence and formation of polymerised formaldehyde in interstellar space. A semi-quantitative explanation for the existence of a low-temperature limit to the rate of growth of chains in the polymerisation of formaldehyde was attained by using the concept of molecular tunnelling in exothermic chemical reactions[14–16]. As can be seen from all the above-mentioned considerations, there are now sufficient grounds to affirm that molecular tunnelling is the only mechanism which can provide the formation of long chains of polymerised formaldehyde in interstellar space.

It would seem most promising and worthwhile to analyse the possible role of molecular tunnelling in the general context of the evolution of prebiotic macromolecules and in the wider field of cosmic organic chemistry with reference to conditions of interstellar space, comets and the early Solar System as well as the primitive earth.

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Dust clouds and frictional generation of glow discharges on Mars

A REMARKABLE characteristic of those samples of the martian soil which have so far been analysed is the absence of carbonaceous matter down to the parts per billion (10^9) level[1]. As well as a lack of endogenous organic material there is no sign of the component expected from infalling carbonaceous meteorites. The inorganic particles constituting the fine martian soil seem to be extremely 'clean'—far cleaner than terrestrial desert or Antarctic analogues. I suggest here that glow discharges generated by friction within dust clouds might explain this apparent absence of carbonaceous matter. In addition glow discharges might account for some reactions noted in the Viking biological experiments.

Fine siliceous material is frequently raised by Martian winds to produce dust clouds, sometimes in enormous quantities sufficient to shroud the entire planet[2]. In the present cold, dry conditions[1] it seems inescapable that frictional electrostatic charging will occur. On Earth we see natural examples of this triboelectric phenomenon in sandstorms and dusty volcanic eruptions[3]. Laboratory studies have confirmed that movement and dispersion of many types of finely-divided material result in the separation of charge and the generation of considerable electrostatic potentials[4]. It is not essential that the system be heterogeneous: homogeneous particles of different sizes can take up opposite polarities. The magnet experiments on the Viking landers have demonstrated a certain heterogeneity in the martian soil[1], however. Separation of the particulate components of a charged cloud can produce oppositely charged regions within it. At the pressures existing in the lower terrestrial atmosphere the potentials may be equilibrated by a spark discharge—the most dramatic example being volcanic lightning[5]. At reduced pressures, however, charge equalisation is facilitated by glow discharge[6]. Experiments[7] show this mechanism to be most effective in the pressure range 0.1–10 torr: at the surface of Mars there is a dry 95% carbon dioxide atmosphere at a pressure of 5–6 torr (7–8 mbar; ref. 1). Studies of the glow discharge resulting from the generation of frictional electricity at low pressures were begun in the eighteenth century[8], but frictional devices were soon supplanted by more reliable and côntrollable sources of high potential for the investigation of electrical discharges in gases. I therefore decided to check experimentally on the effect of agitating dry powders at reduced pressure.

A 5-l Pyrex flask was rotated manually about a hollow stub axle, two O-rings providing a vacuum seal. The speed of rotation controlled the magnitude and form of agitation of the dust from gentle sliding to a continuous cascade, an oscillatory motion proving especially effective. Evacuation was accomplished through the hollow axle by a conventional vacuum system incorporating gauges, a gas bleed, and a two-stage rotary pump. With air as the gaseous medium and fine sand (previously washed and oven dried) as the particulate material it was found that discharges visible in a darkened room were readily excited over a wide range of reduced pressures. At 0.1 torr a faint greenish–white glow extended for more than 5 cm above the agitated sand, being somewhat more intense adjacent to the rising wall of the rotating vessel. The system was then isolated from the pump and air allowed to enter as required through a needle valve. In the pressure range of particular interest (4–6 torr) the continuous glow contracted and brightened, and was accompanied by long, forked, ribbon discharges about 1 cm wide darting intermittently through the cascade of dispersed grains. Occasional intense spots of light on the surface of the moving sand gave a 'twinkling' appearance. At 10 torr ribbon discharges became narrower and brighter, but less frequent. This progression continued until at 50 torr only bright twinkling discharges remained.

It is recognised that the glow discharge is a very effective scavenger of even the most tenacious traces of organic matter from surfaces (particularly silicates) because cleaning with such a discharge is the conventional final stage in preparing an astronomical mirror for aluminising[9]. The mechanism is primarily that of ion bombardment, but reactive intermediates produced in the gas phase may also be important[10]. It is therefore suggested that the fine, near-surface martian soil will have been subjected to repeated dissemination by seasonal winds and, while within these clouds, the separated particles exposed many times to the cleaning action of glow discharges. It will be noted that this process could operate discontinuously throughout an extended period of time right up to the present. It also differs from irradiation by solar ultraviolet in that the latter could not penetrate beyond the surface of a cloud or the undisturbed soil: the two mechanisms may be complementary.

In addition to the cleaning action, it is relevant to consider the accumulated chemical effects which may be produced by glow discharges[7]. It has been shown that they promote dissociation of molecular gases such as carbon dioxide, and can act as effective generators of ultraviolet light, ions, free radicals, atomic oxygen and ozone[11,12]. The last two in particular could persist for varying lengths of time after the discharge, and diffuse to subsurface locations in the grains to exert powerful oxidising effects. Potential products include peroxide bonds, percarbonates (which evolve oxygen and carbon dioxide on gentle warming, and oxygen on addition of liquid water) and hydrogen peroxide addition compounds[11]. It is therefore suggested that the widespread reddish coloration of the martian surface[1], as well as the unexpected reactions observed in the Viking biology experiments[13], could in part be due either directly or indirectly to glow discharges. Living microorganisms (including spore-formers) do not long survive exposure to glow discharges and their products[12], so on this basis the fine, red, surficial martian dust would seem unfavourable for life-detection experiments. A better sampling scheme might be coring in the postulated permafrost terrain[2]. It is perhaps ironic that in different conditions (notably in the presence of water) glow discharges can promote the abiogenic synthesis of organic molecules[7].

Finally, although it is not expected that glow discharges on Mars would be detectable from orbit (much less from Earth), it would seem worthwhile to attempt radiofrequency and electrostatic field measurements on future landings.

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Daytime ionosphere scintillation associated with geomagnetic storms

EQUATORIAL spread F, although a night-time phenomena, has been reported on a few occasions during the dawn period[1]. This is thought to be due to the remains of irregularities produced during the night. Earlier results have also shown that ionospheric scintillations and spread F have

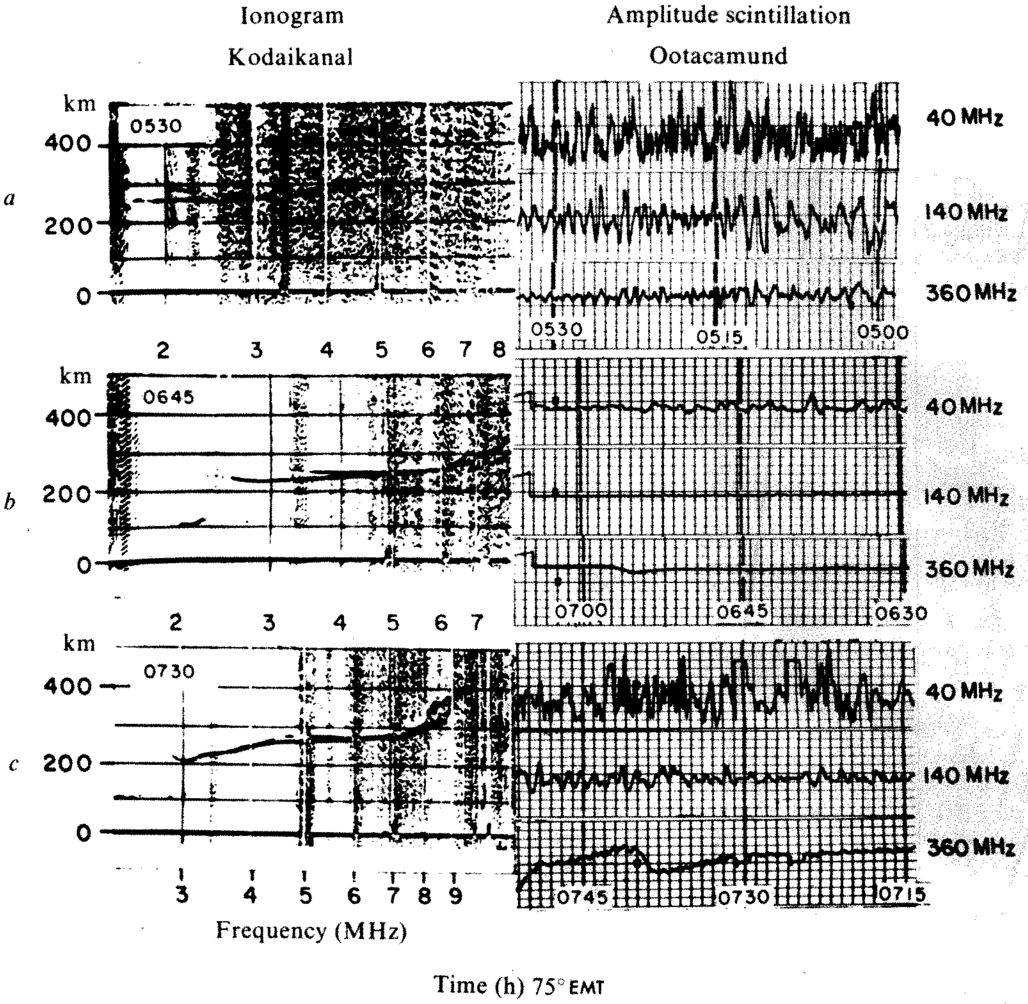

Fig. 1 Ionograms taken at Kodaikanal and the corresponding amplitude recordings of 40-, 140- and 360-MHz radio beacons at Ootacamund on 29 October 1975. Note the association between spread F and ionospheric scintillations specially during daytime (*c*).

a good correlation. These scintillations rarely occur between 0200 and 0800 LT, or around sunset hours[2]. The present article deals with a unique observation of the equatorial region, where the irregularities produced during the night have vanished, and started again after sunrise. The fresh irregularities caused spread F and ionospheric scintillations at 40, 140 and 360 MHz. These irregularities can be associated with a geomagnetic storm.

Figure 1 shows the sample records of the amplitudes of 40-, 140- and 360-MHz beacons as received at Ootacamund (11°4′N, 76°7′E) at 0530, 0645 and 0730 LT (75° EMT). To monitor the ionospheric condition we have compared these records with the corresponding ionograms taken at a neighbouring station Kodaikanal (10°2′N, 77°5′E). During the pre-dawn period on 29 October 1975, both the spread F and the scintillations were quite strong. This is shown by Fig. 1*a*; the scintillation index at 40 MHz was 97%. The spread F as well as the scintillations had disappeared after 0603 LT and one can see in Fig. 1*b* for 0645 LT, clear F traces and fairly smooth amplitude records. There were no more scintillation until about 0712 LT when there was sudden onset on all frequencies and these scintillations continued until 0848 LT. Figure 1*c* for 0730 LT shows spread F near the critical frequency together with scintillations on all the frequencies. No sporadic E irregularities were seen on the ionogram, thus, the scintillations were caused only by F region irregularities.

The Indian Institute of Geomagnetism, Bombay, had observed a gradual commencement type geomagnetic storm at 1800 LT on 28 October 1975. Although the planetary geomagnetic indices were not high on 29 October 1975, the equatorial D_{st} values showed an increase from 1400 UT (1900 LT) to 1800 UT (2300 LT) and a sudden drop afterwards reaching a minimum around 0800 UT (1300 LT). Thus the present observations seem to have occurred during the main phase of a strong geomagnetic storm.

The Faraday rotation angles were recorded at the same time as the amplitudes at Ootacamund. Using these data, total electron contents (TECs) at intervals of 5 min were computed. There were wave-like fluctuations in the TEC in the period of about 10 to 15 min.

Equatorial ionospheric scintillations in the early hours after sunrise is rare. Further, in the present case, the onset at 0712 LT was preceded by a no-scintillation period. Thus the scintillations around 0730 LT were not due to irregularities remaining from spread F of the previous night but were due to newly generated irregularities in the F region.

Various theories[3,4] have been proposed for the generation of spread F near the magnetic equator during the post sunset period. The example shown in Fig. 1*c* is an abnormal case and cannot be explained by normal features of the F region assumed in most theories. It is likely that the F region irregularities discussed here were associated with the geomagnetic storm prevailing during that period.

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Volcanic dust veils and North Atlantic climatic change

One possible climatic control that has been neglected in empirical studies until recent years is the variation in the volcanic dust loading of the upper atmosphere. I shall discuss here the nature of the relationship between volcanic activity and the climate of the North Atlantic sector and its importance in relation to other proposed climatic controls on time scales up to 300 yr. Fluctuations on these time scales in a volcanic dust veil index (DVI) compiled by Lamb[1] and in parameters representative of the strength of the North Atlantic westerly winds and the temperature of central England are shown to be similar.

The DVI is an estimate of the amount of dust in the stratosphere injected by explosive volcanic eruptions. How the amount of dust input from an eruption is assessed depends on the type of observations available. The three methods generally used are based on the greatest depletion of the direct solar beam in middle latitudes, the estimated volume of solid matter dispersed as dust, and the lowering of the average air temperature in middle latitudes by the eruption. All available estimates are finally averaged (Lamb[1] gives further details). The estimation methods give rise to a degree of climatic dependence in the DVI which should not be ignored. The present record contains no assessments based on temperature alone. The atmospheric circulation indices used are temperature-independent.

To examine relationships between volcanic activity, the atmospheric circulation and climate of the North Atlantic sector on timescales of 50 yr or more, non-overlapping 25-yr averages of the DVI, central England temperature[2] (CET) and a record of south-westerly surface wind frequency at London (B. M. Gray, East Anglia, unpublished data; pre 1728 figures are estimates) are used (Fig. 1). The latter is related to the character of the circumpolar westerly upper air flow as it crosses the North Atlantic, where it is determined by the strength, position and orientation of the Canadian upper air trough. These series were selected as they are the longest high resolution records of the atmospheric circulation and climate available for the North Atlantic sector. The degree to which they represent global events is difficult to assess in view of the limited availability of data for other sectors. Trends in CET over the past century are, however, similar to those observed in estimates of global or hemispheric temperature.

Fig. 1 25-yr Averages of *a*, winter temperature in central England; *b*, south-westerly wind frequency at London, provisional values pre-1728; *c*, volcanic DVI.

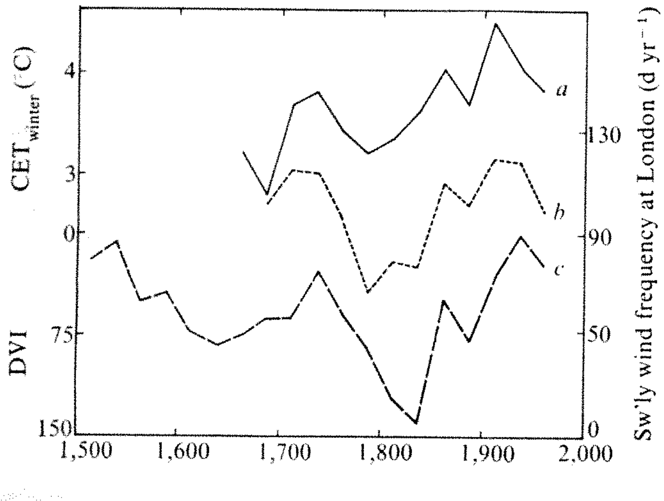


Table 1 lists correlation coefficients between the non-overlapping 25 yr averages of the three records for the period 1725 to 1950. The strongest relationships are between the DVI and the south-westerly frequencies (about 70% of the variance of the annual south-westerly record is explained by the DVI) and between the south-westerly frequencies and CET (about 80% of the $\mathrm{CET}_{\mathrm{w}}$ variance explained by the winter south-westerly frequencies). The input of large amounts of volcanic dust into the stratosphere affects the amount and distribution of solar energy reaching the Earth's surface, thereby influencing the strength of the atmospheric circulation. Lamb[1] showed that the frequency of the North Atlantic upper air westerly winds reduces on a year-to-year timescale following large explosive volcanic eruptions. The strength of these upper air westerlies, and the associated surface advection of air from southerly, oceanic sources, is a major factor in determining the winter temperature in central England and Western Europe.

Table 1 Correlation coefficients for the period 1725–1950 (nine pairs of non-overlapping 25 yr averages).

	DVI	$\mathrm{CET}_{\mathrm{\mu l}}$	$\mathrm{CET}_{\mathrm{winter}}$
DVI		−0.67*	−0.64
South-westerly frequency (annual)	−0.85*	+0.61	+0.86*
South-westerly frequency (winter)	−0.76*	+0.55	+0.90*

*Statistical significance above the 95% level.

Figure 1 shows that three major periods of stratospheric dust clearance have occurred on timescales of 50 yr and more, in the early sixteenth, eighteenth and twentieth centuries. These periods correspond to the maxima in the extended record of CET constructed by Lamb[3] from historical and botanical evidence. The effects of a minor clearance in the second half of the nineteenth century are also clear in the $\mathrm{CET}_{\mathrm{w}}$ and south-westerly frequency records (Fig. 1). Because of the relative shortness of the DVI record, the 200-yr interval between times of major dust clearance is considered fortuitous rather than evidence of a predictable cycle. A climatic cycle of about this length has, however, been frequently linked to solar variation[4,5]. Roosen *et al.*[6] proposed that this periodicity might result from regular changes in the moon's orbit. The associated changes in tidal stress could trigger volcanic activity and hence affect climate.

The varying level of volcanic activity explains much of the variation in CET on time scales of 50–300 yr but certain fluctuations present in CET are not seen in the DVI. Although the 200-yr periodicity is present in all three records, only $\mathrm{CET}_{\mathrm{w}}$ contains a linear trend (Fig. 1). This trend results from the recovery from the depth of the Little Ice Age which occurred during the second half of the seventeenth century. Neither the magnitude of the DVI, nor the reduction of the south-westerly wind frequencies is sufficient to account for this climatic severity. Although all three records must be considered suspect during this period, another climatic control, perhaps affecting

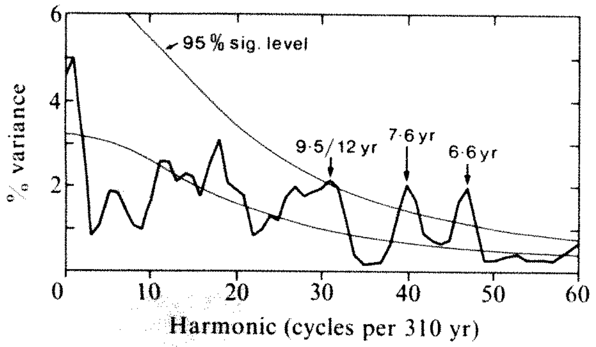


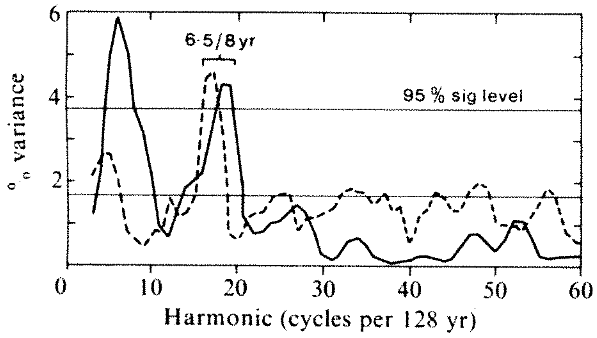
Fig. 2 Variance spectrum of the DVI, data for period 1500–1966. Calculated by taking the Fourier transform of the autocorrelation function, truncated at one-third of the data length. Red noise null continuum assumed as lag one autocorrelation coefficient significantly different from zero.

some other component of the atmospheric circulation, is required to explain the severity of this climatic shift. The low level of solar activity during the Maunder Minimum[7] of the seventeenth century may have been responsible. Solar particles emitted during solar flares seem to influence the strength of the meridional component of the North Atlantic atmospheric circulation[8,9], although the mechanism is unclear. The climatic effects of changes in the meridional circulation of the atmosphere are generally obscured as the zonal circulation exerts a stronger control over the UK climate[9]. Schneider and Mass[10] have modelled fluctuations in global temperature since 1600 employing fluctuations in volcanic and solar activity as climatic controls. In addition to solar variability, human activity may be a significant factor from the mid-twentieth century onwards.

Explosive volcanic eruptions inject into the stratosphere large quantities of volcanic dust which slowly falls out over succeeding years. This gives the annual DVI record a saw-toothed appearance: a series of impulses which gradually decay. Because of the damped response of the atmosphere–ocean system, a succession of fairly regular volcanic eruptions may result in an apparently cyclical climatic fluctuation. If volcanic eruptions occur with approximate regularity, a corresponding peak should appear in the variance spectrum of the DVI. If volcanic dust is an important climatic control, a similar peak may also appear in the spectra of climatic data for the same period. Matching peaks, however, do not necessarily show that volcanic activity and climate change in a regular, predictable cycle, the DVI periodicity may just be a chance characteristic of the data interval employed.

The variance spectrum of the entire DVI record is shown in Fig. 2. Three peaks are significant at the 95% level: at 9–12.5 yr, 7.6 yr and 6.6 yr. The peak at 9–12.5 yr is broad and, therefore, represents a process of highly variable period. It will not be discussed further. Bandpass filtering indicates that the two cycles at 7.6 and 6.6 yr are present through most of the period 1500 to 1966 but of low amplitude during the eighteenth century. They interfere to produce an amplitude modulated cycle with a wavelength close to 7 yr. The interval between periods of maximum amplitude is about 50 yr. The frequency and phase of the cycle are also variable. The fact that the parameters of the cycle (amplitude, phase, period, etc) are not constant places a considerable restriction on its forecasting potential.

Fig. 3 Variance spectra of the DVI (————), data for period 1850–1966, and the P index (– – – –), data for period 1861–1971. Calculated using the fast Fourier transform, to obtain similar resolution and avoid data loss.



The P index is a measure of the mobility of the flow of the atmospheric circulation over the UK on a day to day time scale[11]. In general, a high value of the P index indicates a period of mobile, westerly and cyclonic circulation types associated with vigorous North Atlantic westerlies. The variance spectra of this index and the DVI, calculated from available data for the period 1850 to date, are shown in Fig. 3. Both spectra contain a statistically significant peak at 7–8 yr. Considering the resolution of these spectra, the two peaks are indistinguishable in period. The variance spectrum of the CET record for the period 1816–1973 contains a similar peak but the earlier section of the record (1659–1815), comprising the eighteenth century when the 7 yr cycle in the DVI was of low amplitude, does not. Berlage[12] has discussed the apparently global extent of the 7-yr climatic cycle which is particularly marked in the El Niño phenomenon on the coast of Peru. However, many claims made for climatic cycles of any period are based on doubtful statistical analysis and require re-examination with modern techniques.

The volcanic cycle of 7 or 8 yr, and the associated climatic variation, may possibly be forced and, to a certain extent, predictable, as a similar fluctuation is present in parameters of the earth's rotation. Anderson[13], and Press and Briggs[14], have suggested that variations in seismicity, the two components of the earth's rotation (the rotational speed and polar motion) and the atmospheric circulation (and climate) could be linked. A fluctuation of 7 or 8 yr has been observed in modern measurements of the Earth's rotational speed[16], although the data interval is insufficient to test its statistical significance. Interaction between the annual component of the polar motion, forced by seasonal redistribution of the atmospheric mass, and the Chandler Wobble period of about 14 months gives rise to an approximately 7-yr fluctuation in the polar motion. The amplitude of the annual component also undergoes a 7-yr fluctuation[16]. It has been claimed that the polar motion could have a tidal influence on the atmosphere[16]. It is dangerous to discern cause and effect in such fragmentary evidence but the possibility that volcanicity, seismicity, the atmospheric circulation and the earth's rotation form a complex, interactive loop with a characteristic timescale of about 7 yr, perhaps determined by the period of the Chandler Wobble, cannot be neglected.

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Oxygen isotope stratigraphy of Late Pleistocene coral terraces in Barbados

DISPUTE over the correlation of the last interglacial event in deep-sea cores with the corresponding shoreline feature on islands preserving fossil coral terraces has become a fundamental stumbling-block to progress in Pleistocene geochronology. If polled independently most scientists working in this area would agree that the last interglacial event is clearly recorded in their own data, yet the problem of precise correlation among the various types of data has remained the subject of controversy for the past eight years[1-8]. By making oxygen isotope determinations in molluscs collected from the Late Pleistocene coral terraces in Barbados, we have directly correlated these terraces with the oxygen isotope stratigraphic record of deep-sea cores, and have established that Barbados terraces I, II, and III (ages 82,000, 105,000 and 125,000 yr BP) were all formed during stage 5 of the standard oxygen isotope stratigraphy.

We will first review the data from interglacial highstands, and then that from interglacial sections in deep-sea cores. Pleistocene beach deposits are widespread on all the continents and on many islands; of particular interest are the latitudes of coral growth, within which Pleistocene sea-level stands are marked by fossil coral terraces. Aragonite coral skeletons are amenable to absolute dating using both the ^{230}Th/^{234}U method[9] and the ^{231}Pa/^{235}U method[10] within the time range Recent to ~ 150,000 yr BP. A large number of localities possess coral reef terraces which yield uranium-series dates in the region of 125,000 yr BP[4,11,12]. In tectonically stable areas, such as the Bahamas[13], Hawaii[14] and elsewhere, the 125,000 yr old terrace is found several metres above present sea level, and is the youngest pre-Holocene terrace present[9]. These data indicate that the 125,000 yr BP sea was above the present sea level, and that since that time the sea has been continuously below its present level.

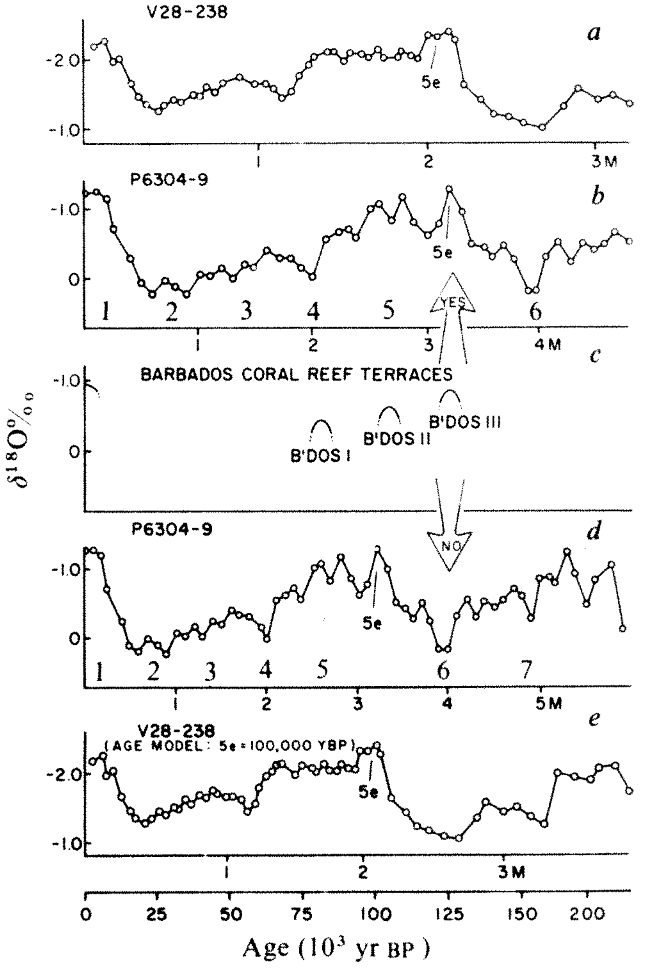
Coral reef sequences from tectonically emergent areas support the inference that subsequent sea level maxima have not reached the present level. For example, on Barbados, the Barbados III shoreline (125,000 yr BP) is well mapped and abundantly dated. Owing to tectonic uplift, the feature is presently at elevations ranging from 20 to 60 m; at lower elevations the Barbados II and Barbados I terraces are also mapped and dated[2]. Sea levels considerably below the present level have been estimated for these times by assuming a constant uplift rate calibrated by the present position of the Barbados III terrace. Similar studies in New Guinea on the tectonically emergent Huon peninsula lead to the same conclusion[15], as do studies in the Ryukyu Islands[11]. This implies then that the 125,000 yr BP terrace records the last interglacial in the sense that it formed the last time that continental ice volume was as small as it is today.

In deep-sea sediments, the oxygen isotopic composition of calcareous foraminifera has become a widely used stratigraphic tool. The oxygen isotope record has been divided into isotope stages whose boundaries can generally be easily recognised in all oceans[16]. Stage 5 was the last predominantly interglacial stage[3,4,17], within which the earliest substage 5e may probably be correlated with the last interglacial in a more strict sense as used, for instance, by palaeobotanists[3]. The stage 6–5 boundary, also known as Termination II[4], has been described as the most widely correlatable horizon in the whole geological column[18]. It is the age of this clearly-defined horizon, or of the equally clear interglacial event which followed it, that is a matter of contention.

One very persuasive line of reasoning, stems directly from the interpretation of the oxygen isotope record. It has been argued that the changing oxygen isotopic composition which is observed in foraminifera from deep-sea sediment cores, stems almost entirely from changes in the oxygen isotopic composition of the ocean itself, resulting from the storage of isotopically light water in the continental ice sheets[16,19-21], and hence may be an ice-volume or sea-level record. Clearly, if this is a correct interpretation, then there can be no doubt as to the correlation of the Barbados III terrace, with the earliest part of stage 5, which was uniquely interglacial in isotopic terms[3]. There are, however, reasonable arguments in favour of a smaller contribution to the oxygen isotopic record from this source[22], and if they are followed then the correlation of the Barbados III coral terrace with the early part of stage 5 is not a unique solution.

An alternative approach, of course, is the dating of the marine sediments themselves; this is the chief source of evidence for an alternative chronology. Beyond the range of ^{14}C dating, the uranium decay series may be used to date marine sediments. The application is less simple, however, than in the case of coral dating; there are difficulties in the analytical methods and acute problems regarding the interpretation of the measurements[24] which are widely acknowledged to exist. Several cores have yielded apparently consistent data which clearly point to an age near 90,000 yr BP for substage 5e[25]. Another core has been studied in considerable detail, and has consistently yielded an age near 120,000 yr BP for the same event[2,4,27]. Few other cores have been analysed in sufficient detail to contribute to a solution of the problem, and interlaboratory comparisons have so far only clouded the issue[28]. Thus, if correlation is to be based only on an absolute age estimation, a case may be made for correlating substage 5e either with the Barbados II or the Barbados III terrace.

Fig. 1 Oxygen isotope stratigraphy of Barbados coral reef terraces (*c*) compared with oxygen isotope stratigraphy of Caribbean core P6304-9 (*b* and *d*) and of equatorial Pacific core V28-238 (*a* and *e*). All five are plotted against a time axis; for the cores, depth in metres is also indicated. *a* and *b* are plotted assuming that the age of substage 5e is about 125,000 yr BP; *d* and *e* are plotted assuming that the age of substage 5e is about 100,000 yr BP. In *c*, the oxygen isotope data from this paper are plotted at the ages determined for the coral terraces from which our samples were collected. Data for *a* and *e* in refs 3, 4, 17 for *b* and *d* from ref. 31; for *c* from Table 1. The alternate timescales indicated respectively by *a* and *b*, and by *d* and *e*, are those depicted in Fig. 4 of ref. 8.



This dilemma, as well as other less substantial evidence, led Emiliani and Shackleton[8] to publish their infamous 'double curve' in which generalised versions of the isotopic record as a function of time were sketched for two different timescales through the last interglacial.

Clearly, the correct way to solve this problem is to make a direct stratigraphic correlation between the coral reef sequence and the deep-sea sediment sequence, instead of attempting correlation by means of age estimation. The coral terraces of Barbados contain molluscs which, like foraminifera, deposit their carbonate shells in oxygen isotopic equilibrium with sea-water[23]. Thus, the oxygen isotope record in a mollusc sequence should, in principal, be almost identical with the sequence observed through isotopic analysis of planktonic foraminifera in deep-sea sediments from the nearby ocean floor. Specifically, molluscs in the Barbados III terrace should be 0.1 ± 0.1 per mil more isotopically negative than those living today, if correlation with 5e is correct[3] and if 5e is therefore 125,000 yr old; they should be up to 1.8 per mil more positive if the terrace formed during the cold part of stage 6 (Fig. 1,) and 5e is correlated with Barbados II with an age of about 100,000 yr; they should be about 0.9 per mil more positive, with the age of 5e being about 90,000 yr, according to the correlation shown by Emiliani and Rona (they remark that molluscs in some raised beaches do give isotopically positive ^{18}O values suggestive of a glacial age—see ref. 7).

Molluscs were collected by R. K. Matthews from Barbados terraces I, II and III during summer 1973. Care was taken to collect only from reef front localities (predominantly *A. palmata* and *A. cervicornis* facies). By specifically avoiding lagoonal materials, we avoid the anomolous salinity and temperature conditions characteristic of that environment. Modern subtidal molluscs from Barbados were collected alive specifically for this study by Finn Sander (Director of the Bellaires Research Institute, Holetown, St James, Barbados W1).

Our first isotopic measurements on these materials was made in Cambridge[8] during 1974. Shells were sampled as described in ref. 30, and analysed in a VG-Micromass 602C mass spectrometer using the sample preparation system described in ref. 16. In the course of these determinations and subsequent investigation of the specimens in thin section, we became somewhat apprehensive about possible contamination by boring algae. We evolved a new sampling procedure. At Brown, molluscs were embedded in plastic, sectioned perpendicular to the shell wall and polished. An acetate film peel replica of the polished surface was prepared before and after sampling the shell with a small drill or with a tungsten carbide scriber. Examination of the peels with a light microscope allows detection of any areas of alteration or other abnormalities which may be avoided in sampling. Subsequent peels provide a permanent record of precisely what was sampled. We repeated the isotopic analyses at Brown University during 1975. The analytical methods used in the Benedum Stable Isotopes Laboratory at Brown University were similar to those used in Cambridge; again, a VG-Micromass 602C mass spectrometer was used. Results of these two independent studies are substantially in agreement. We report here only the second data set because we are confident that our new sampling procedure avoids contamination from boring algae.

Variation in average oxygen isotopic composition among Barbados Recent, Barbados I, II, and III are summarised in Table 1. We consider variation in average isotopic composition among these stratigraphic units to be primarily a measure of differences in global ice volume. We have, however, given detailed consideration to the possible contribution of temperature and salinity variations. The seasonal isotopic ranges measured are in good agreement with the range of values we observe in recent molluscs. The potential contribution of temperature and salinity to variation among the means is small. More importantly, these factors would also affect isotopic compositon of planktonic foraminifera from tropical deep-sea cores. As this paper deals with correlation of Barbados terraces to deep-sea cores by means of oxygen isotope stratigraphy, the precise origin of the signal which is common to the two data sets is of secondary consideration.

Table 1 Average oxygen isotopic composition of Barbados molluscs from Recent and Pleistocene terraces. Values are reported in standard notation relative to the PDB standard[23]

Stratigraphic level	δ^{18}O mean and standard deviation (‰)
Living Recent (17 analyses among 6 specimens)	−0.93 ±0.26
Barbados I (82,000 yr BP) (23 analyses among 9 molluscs)	−0.41 ± 0.41
Barbados II (105,000 yr BP) (16 analyses among 6 molluscs)	−0.62 ±0.40
Barbados III (125,000 yr BP) (16 analyses among 6 molluscs)	−1.04 ±0.55

One sample taken from one of the Barbados III shells yielded a value of +1.77‰, well over three standard deviations from the mean. We have not included the measurement in estimating the mean for this terrace. The observed standard deviation derives partly from seasonal temperature variation at the site; if average temperature varied with a standard deviation of ±1 °C this would give rise to oxygen isotopic composition varying with a standard deviation of ±0.2‰. Data will be submitted to the CLIMAP Data Base Management System at Brown University and subsequently archived with the National Geophysical and Solar-Terrestrial Data Center (NGSDC), Boulder, Colorado.

Figure 1 presents graphic comparison of the oxygen isotopic record of two deep-sea cores and the Barbados terraces in the time range 0–150,000 yr BP. Graphs *a* and *b* place isotopic substage 5e at 125,000 yr BP whereas graphs *d* and *e* place substage 5e at 100,000 yr BP. Graph *c* presents Barbados mollusc isotopic data from Table 1 and terrace ages as determined by ^{230}Th dating of corals from Barbados I, II, and III. Correlation of the Barbados data with graphs *a* and *b* is good, whereas correlation of Barbados data with graphs *d* and *e* forces correlation of isotopically light (interglacial) Barbados III with outrageously glacial conditions in deep sea cores. Thus, we conclude that isotope substage 5e must surely be correlated with Barbados III and must, therefore, in turn be correlated wtih the terraces elsewhere in the world which indicate a sea-level somewhat above present levels at around 125,000 yr BP. While geochemists may continue to discuss the relative precision of absolute age estimates obtained from coral terraces and from deep-sea cores, our data firmly establish the stratigraphic correlation between the two sequences.

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Biological markers in coal and coal liquefaction products

ISOLATION of saturated isoprenoids from coal liquefaction products is an indication of the remarkable stability of these compounds. In the present investigation we show that some 'biological markers' and asymmetric carbons can survive the conditions of coal liquefaction without rearrangement or complete racemisation. Also, odd carbon numbered *n*-alkanes do not predominate, as reported for some coals, in either the coal liquefaction products studied or the feed coal. The distribution of saturates in the synthetic oil is very similar to that of some petroleums[1]. The detection of 'biological markers' in coal and coal liquefaction products supports the geological and chemical evidence that coal is a product of the progressive fossilisation of plant debris. Optically active materials isolated from peat, lignite, brown and bituminous coals corroborate this theory. Additionally, hydrocarbons bearing skeletal structures that are widely distributed among plants have been extracted from coal. Ōuchi and Imuta[2] have found normal alkanes from C_9–C_{31} in the benzene extracts of Yūbari coal; more recently, Harrison[3] and Bartle, *et al.*[4] have identified the normal alkanes and saturated isoprenoids in the supercritical toluene extracts of bituminous coals.

'Biological markers' are compounds which exhibit long term chemical stability to diagenesis and which also possess skeletal features related to biosynthetic sequences[5]. Compounds such as *n*-alkanes, iso-alkanes, anteiso-alkanes, isoprenoid alkanes, porphyrins, and long chain fatty acids show stability for long time periods in geological conditions and have been identified in coals[4,6], petroleums, oil shales, recent and ancient sediments[1], meteors, and oil sands. Also, the observation of optical activity among organic compounds is closely associated wtih their biosynthetic origins.

During an investigation of the saturate materials in coal liquefaction products, several classes of compounds that are biological markers were detected. The oil studied was produced by catalytic hydrodesulphurisation of coal at 450 °C and 300 atm pressure of hydrogen in a half-ton-per-day bench scale SYNTHOIL unit at the Pittsburgh Energy Research Center[7,8]. In this investigation, the solids in the gross liquid product were removed by centrifugation in the SYNTHOIL plant and the resulting liquid subjected to solvent separation using benzene and hexane[9] to yield a heavy oil constituting 60–70 wt % of the centrifuged liquid product. The heavy oil fraction was separated by the SARA[10] (saturates, aromatics, resins, and asphaltenes) technique to yield a saturate fraction which amounted to 6–7 wt % of the heavy oil. The saturate material was further separated on 5-Å molecular sieves[11] to yield *n*-paraffin and non *n*-paraffin fractions, 29 and 71 wt % of the saturate fraction, respectively. These fractions were then chromatographed and analysed by combined gas chromatography–mass spectrometry (GCMS).

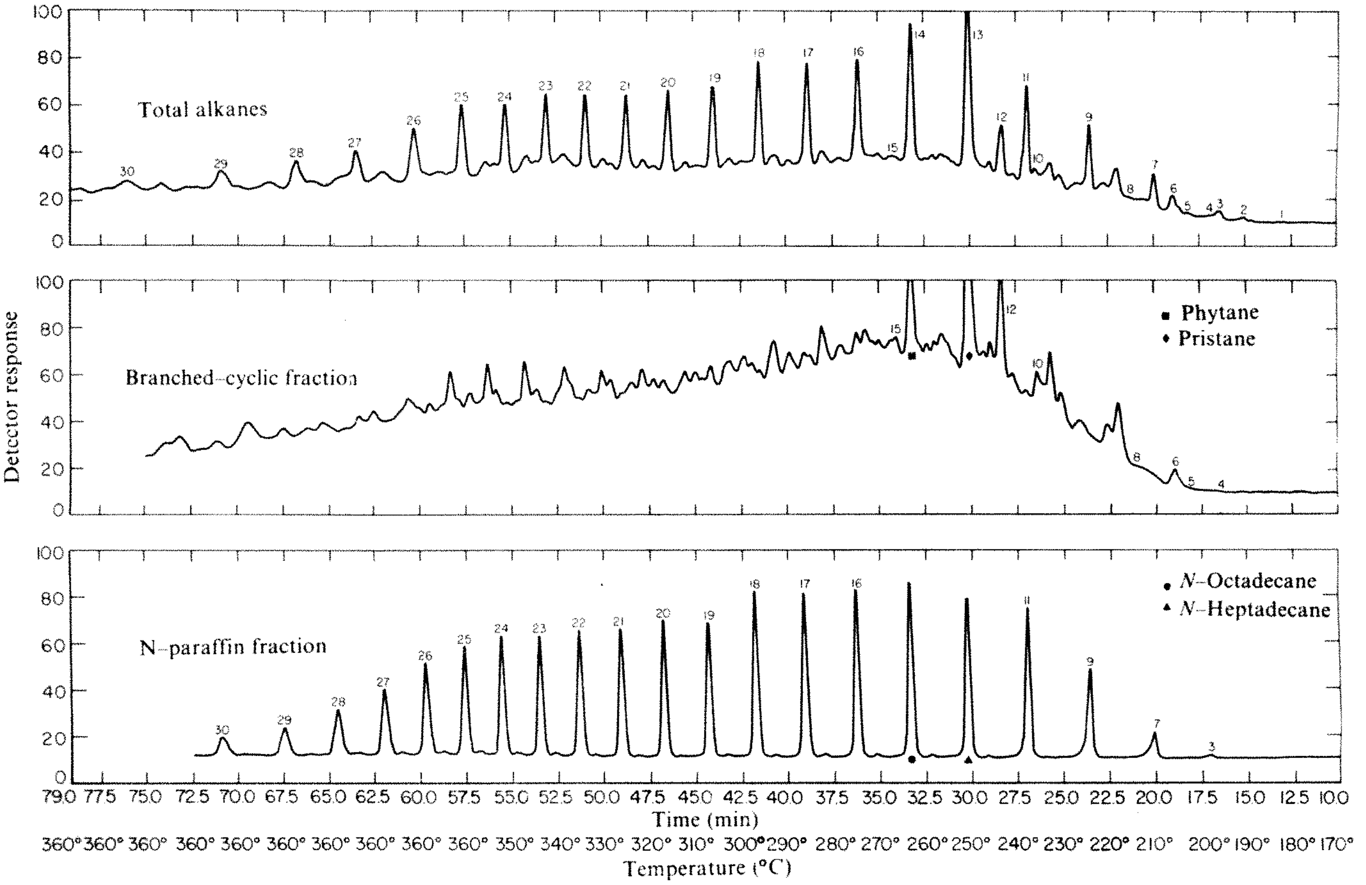

Fig. 1 The top chromatographic profile is of the total saturate materials isolated from coal liquefaction product. The middle and bottom chromatograms are subfractions of the total saturate materials separated by 5-Å molecular sieves. The combined GCMS analyses were performed with a DuPont 490 mass spectrometer interfaced to a Varian 1700 Series gas chromatograph, equipped with an 80:20 splitter and a flame ionisation detector, and to a Hewlett-Packard 2100A computer used for spectrometric data storage and reduction. The gas chromatographic separations were performed employing a 15 feet × ⅛ inch stainless steel column packed with 5% Dexsil 300 on acid washed Chromosorb-G at a helium flow rate of 30 cm^3 min^{-1} and appropriate temperature programming conditions. Each of the above chromatograms were run in duplicate. In all cases, the two chromatograms of the same material were identical.

Table 1 Analyses of the total saturate materials from a coal liquefaction product

GC peak number	Compound*	saturates† (mg per g)
1	*n*-dodecane	trace
2	C_6-cyclohexane‡	trace
3	*n*-tridecane	4.3
4	C_3-decalin	0.7
5	methylethyldecalin	1.1
6	cyclohexylcyclohexane	3.9
7	*n*-tetradecane	8.7
8	methylcyclohexylcyclohexane	0.9
9	*n*-pentadecane	12.0
10	perhydro-3-ring	1.4
11	*n*-hexadecane	13.4
12	C_{18}-isoprenoid	6.9
13	*n*-heptadecane and pristane	50.1
14	*n*-octadecane and phytane	38.7
15	perhydropyrene	3.6
16	*n*-nonadecane	17.2
17	*n*-eicosane	15.5
18	*n*-heneicosane	15.7
19	*n*-docosane	14.3
20	*n*-tricosane	14.3
21	*n*-tetracosane	12.2
22	*n*-pentacosane	11.5
23	*n*-hexacosane	12.2
24	*n*-heptacosane	10.1
25	*n*-octacosane	10.7
26	*n*-nonacosane	10.4
27	*n*-triacontane	9.0
28	*n*-hentriacontane	8.9
29	*n*-dotriacontane	5.3
30	*n*-tritriacontane	3.4
		316.4 mg

*Personal communication, D. M. Jewell: many of the low boiling materials are lost during the solvent removal steps of the SARA fractionation. The loss is essentially zero for hydrocarbons of 15 carbons and above.

†The quantity of the various hydrocarbons listed is approximately within ±20% of the amount shown.

‡Indicates the number of alkyl carbons as substituents on the ring.

The chromatographic profiles of the saturate materials isolated from the coal liquefaction products before and after separation into two fractions on 5-Å molecular sieves are shown in Fig. 1; the identifications of the various numbered chromatographic peaks are given in Table 1. Inspection of the chromatographic profile of the *n*-paraffin fraction from the coal liquefaction product indicates that the odd carbon numbered *n*-paraffins do not predominate over the even. The carbon preference index[1], CPI, calculated using mole per cent data obtained from the chromatographic profile of the *n*-paraffin fraction equals 1.08. The *n*-paraffins are equally distributed between odd and even carbon numbered homologues. Others[12] have shown that thermal alteration of organic sediments causes elimination of any carbon numbered preference. Thus, if the feed coals contained *n*-alkanes having a large predominance of odd carbon numbered homologues, liquefaction could eliminate this predominance. Preliminary data indicate that the distribution of *n*-alkanes in this coal liquefaction product is similar to their distribution in some petroleums[1].

A sample of the feed coal, 364 g, used in the liquefaction process (Ireland Mine, W. Va., hvAb) was extracted in a soxhlet using benzene, in order to show that some of the *n*-paraffins found in the liquefaction product were originally in the feed coal. The benzene was removed from the extract by freeze drying, yielding 1.72 g of solids. This solid was batch extracted with *n*-pentane and centrifuged, yielding 1.29 g of pentane soluble material. The pentane soluble material was batch washed through silica gel (Brockman Activity I, 70–230 mesh), yielding 0.33 g (0.99 wt % of original coal) of non-silica gel-absorbed material. The pentane-soluble, non-silica gel-absorbed material was analysed for the *n*-paraffins by combined GCMS. The chromatographic profile and identification of the normal paraffins is shown in Fig. 2. Inspection of this chromatogram indicates that odd carbon numbered *n*-paraffins do not predominate over even. The *n*-heptadecane peak also contains pristane. Ōuchi and Imuta's[2] data, CPI ~1.09, indicate that there is no predominance in Yūbari coal and thus, is in agreement with the results obtained in this investigation, while the recent work of Bartle, *et al.*[4] indicates that there is a predominance of odd over even carbon numbered *n*-paraffins in the bituminous coal they studied. Leythaeuser and Welte[13] have clearly shown that, in the case of coals from the Upper Carboniferous of the Saar district, the predominance of odd over even carbon numbered *n*-paraffins decreases with increasing coal rank. This explains why the results among different workers concerning the predominance of odd over even carbon numbered *n*-paraffin homologues in coal seem to disagree. Bartle, *et al.* investigated a low rank coal (NCB Coal Rank Code 802) while the coals investigated here and by Ōuchi and Imuta were high rank bituminous. The work of Leythaeuser and Welte included only coals of specific European origin and, therefore, a more comprehensive study involving many coals from different continents is needed to determine whether the correlation between *n*-paraffin distribution and coal rank can be generalised.

In addition to *n*-alkanes, discussed above, another class of biological markers found in coal liquefaction products is the saturated isoprenoids—pristane, phytane and a C_{18} isoprenoid were identified. The precursors of these hydrocarbons are unknown, but it is surmised that they result from the diagenesis of phytol, a moiety widely distributed among plant pigments. Phytane and pristane are known to be in coals[2,3]. Thus the saturated isoprenoids and normal paraffins found in coal liquefaction products may be from two sources: (1) formed during the liquefaction process from a precursor having the same or similar carbon skeleton, or (2) are present in the feed coals as entrained alkanes extractable by benzene, survive the liquefaction process, and go into the liquefaction product.

The optical activity of the saturate fraction from a coal liquefaction product was investigated and was found to rotate plane polarised light, $[\alpha]_D^{25} = +0.340°$. The low degree of optical rotation is possibly a consequence of the partial racemisation of many enantiomers during the liquefaction process. It is interesting that the saturate fraction was dextrorotatory. Zahn, *et al.*[14,15]

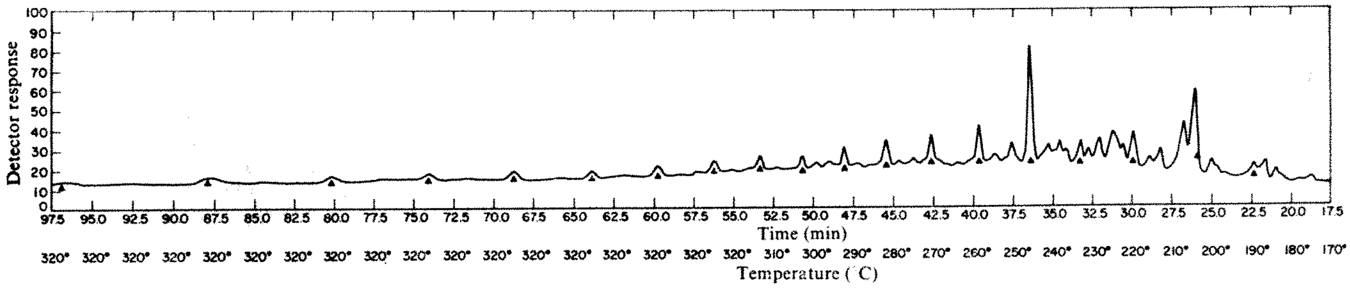

Fig. 2 Chromatographic profile of the pentane-soluble, non-silica gel absorbed material obtained from the benzene extraction of hvAb coal. The starred peaks indicate the *n*-paraffins. The first such labelled peak is *n*-tridecane, the second is *n*-tetradecane, and so on. The analysis was performed using the same equipment and conditions described in Fig. 1. This material was gas chromatographed twice to minimise errors in sample handling. Both chromatograms were identical.

showed that the distillates from coal hydrogenation products and carbonisation tars from Pittsburgh seam coals showed positive rotation only. Hydrogenation oils produced from other coals displayed various positive and negative rotations but the distillates having positive rotation were far more numerous than those having negative rotations[14,15]. Mair[16] has made the same observation concerning petroleum distillates.

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Stratigraphy of buried soil at Teindland Forest, Scotland

EDWARDS *et al.*[1] have described and discussed new palynological and fabric analyses which have made an important contribution to our understanding of the stratigraphical history of the unique buried soil at Teindland, first described and dated by Fitzpatrick[2]. Since 1962 detailed information relating to the distribution and periglacial modification of glacial deposits in the vicinity of Teindland has been gradually accumulated during the soil survey of the area represented on Sheet 85 (Rothes)[3]. Here we relate these field observations to the stratigraphy of the buried soil section at NJ 297570, and obtain a rather different interpretation of the nature of the 'sandy till' described by Edwards *et al.* as overlying the buried soil, and of the general stratigraphy of this site.

The summit area of Teindland Forest and the east facing slopes are covered by till and solifluction deposits derived from sedimentary rocks of Middle and Upper Old Red Sandstone age with some admixture of acid schists in the till at the southern end of Teindland Forest. Along the lower edge of the east facing slope there are scattered remnants of sandy and gravelly lateral moraine mostly lying above the line of the B9015 road. Between junctions at NJ 311522 and NJ 309550 this road also marks the approximate upper edge of the highest fluvioglacial terrace of the Spey Valley.

Above about 185 m no frost cracks are present in the compact red till exposed in profile pits and natural sections. On the eastern sideslopes the till is generally overlain by a very variable blanket of coarse textured stony solifluction deposits and below 120 m distinctive grey frost cracks with ochreous edges are present in the till. This distribution pattern has been confirmed at Wood of Cairnty on the east side of the Spey Valley. In sections alongside a forest track at about 165 m only the remnant basal tips of frost cracks can be seen, whilst downslope in a roadside cutting at about 125 m well-developed frost cracks are present in red till which is buried below a surface layer of solifluction debris. Most of the localities around Teindland where frost cracks or wedges have been recorded during soil survey are listed in Table 1.

From the field evidence it is clear that at some time after the deposition of the local red till there was a period of intensely cold periglacial climate during which a fairly uniform pattern of frost cracks and wedges was developed over the local landscape. Subsequent denudation of the hilltops with downslope migration of solifluction debris was not necessarily restricted to one episode. There is considerable evidence[4,5] for widespread solifluction and erosion in Scotland during the period of the Loch Lomond Readvance (Pollen Zone III)[6] and local evidence of some later erosion in the form of footslope accumulations of sandy gravel and colluvium above a peat band containing a late Sub-Boreal pollen assemblage at Bogincur farm (NJ 307554).

The pit face at Teindland has eroded considerably since it was first described. As exposed in 1962 the organic horizon, subsequently ^{14}C dated at 28,140+480 and −450 years b.p. (NPL−78), had the form of a solifluction lobe cut through by a number of stepped frost cracks (Fig. 1). Only a limited part of the buried organic A horizon remains and on the longer backslope face of the pit the buried soil is represented by a layer of yellow-brown sand derived from the B horizon.

Thin sections prepared from the organic horizon in 1965, 1967 and 1976 consistently show that the organic material is heavily charred and contains coniferous charcoal closely resembling *Pinus sylvestris* charcoal prepared from wood recovered from a 'Grentz' horizon[7]. The presence of (burnt out) pine wood in this context may be indicative of the onset of colder climatic conditions[8]. The charred nature of the organic horizon and the distinctive sandy infill of the cracks suggests that the sand above the organic horizon was deposited soon after the fire. Photographs taken in January 1977 show clearly that this sand layer constituted part of the curved front of a solifluction lobe low down on the eastern side of the present section and was therefore deposited before this lobe formed. The size of the frost crack steps relative to the size of the solifluction lobe (Fig. 1) indicates some movement of the lobe before as well as after the formation of the cracks, so that permafrost was already present before the cracks formed. West[9] has noted that temperatures around −6 to −8 °C, considerably lower than the temperature required for the promotion of permafrost, seem to be necessary for the formation of frost wedges. The surviving part of the buried H horizon seems to owe its preservation to rapid burial (at a break of slope) under sand eroding from the A and B horizons of the surface immediately upslope, a cover which was rapidly consolidated by the subsequent rise of the permafrost table. The material overlying the organic layer, described as 'sandy till' by Edwards *et al.*[1], is similar to but slightly coarser in texture than, the red till generally encountered in profile pits close to this site (Table 1). The very variable nature of this deposit which has been revealed over a period of years and the included irregular sandy lenses are not typical of the local red till. The

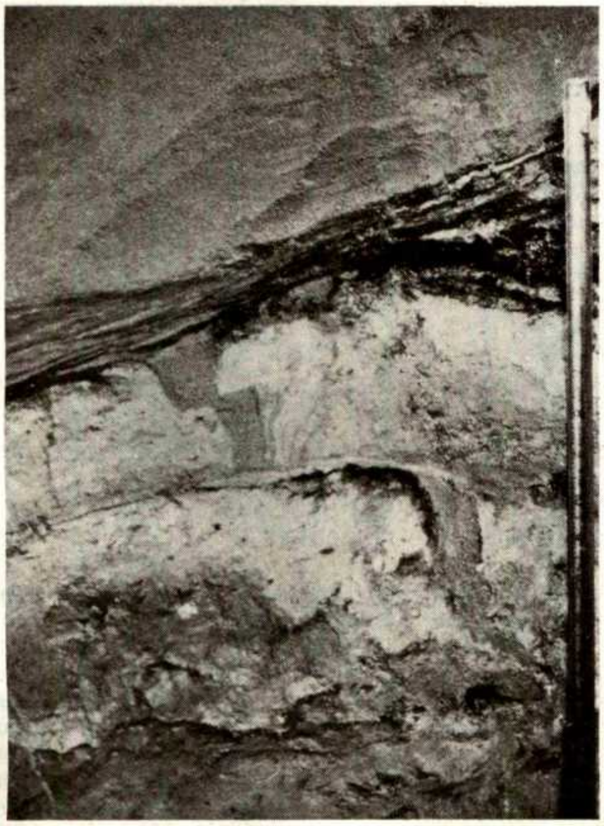
Table 1 General distribution of periglacial phenomena around Teindland, range in clay content of the local red till and clay content of soliflucted till above the approximately 28,000 years b.p. organic horizon at Teindland.

Site name	Description	Grid reference	Altitude* (m)	Presence/absence of frost cracks/wedges	Clay content of till (%)
Findlay's seat no. 1	Profile pit	NJ 283 537	260	Frost cracks absent	10–16
Teindland forest no. 1	Profile pit	NJ 289 548	240	Frost cracks absent	11–17
Teindland no. 2	Profile pit	NJ 300 529	185	Frost cracks absent	≈14
Teindland seed beds	Profile pit	NJ 295 569	125	Frost cracks present 38–104 cm+	≈20
Clatteringbriggs wood	Profile pit	NJ 290 581	85	Frost cracks present 48–118 cm+	12–15
	Test hole	NJ 296 580	85	Frost cracks present below 33 cm	–
Red burn	Bank section	NJ 302 576	75	Frost cracks present 56–180 cm	–
	Gravel pit	NJ 310 552	60	Several frost wedges present	–
	Gravel pit	NJ 307 545	90	Frost wedge present	–
	Gravel pit	NJ 306 518	120	2 m frost wedge present	–
	Gravel pit	NJ 297 510	85	Small frost wedge present	–
Wood of Cairnty	Forest track section	NJ 334 533	165	Basal tips of frost cracks present	12–16
Wood of Cairnty	Roadside cutting	NJ 343 520	125	Frost cracks present in till underlying a solifluction deposit	–
Buried soil section at Teindland	Quarry pit	NJ 297 570	100		7–9

*Altitudes estimated to nearest 5 m by interpolation between contour lines of Ordanance Survey 1:25,000 scale map.

absence from this deposit of a regular pattern of polygonal frost cracks as seen in the red till in nearby exposures along the Red Burn (Table 1) (which would clearly have been just upstream of the Teindland site during any Devension glaciation) confirms that this 'sandy till' is in fact soliflucted till and lateral moraine derived from the nearby slopes of Teindland Forest. This interpretation is consistent with both the pollen data and particle size and shape criteria indicative of glacial origin presented by Edwards *et al.* The preferred orientation of pebbles of 110–120° measured by Edwards *et al.* is not in accordance with the orientation to be expected from free downslope flow of a solifluction deposit[10], but it should be pointed out that this measurement was made only a few metres from the position at which the downflow must have stopped at the change of slope. Pebble orientation at this point is, therefore, unlikely to be in the direction appropriate to unconfined free flow, and orientation in concordance with ice flow direction is coincidental.

Fig. 1 Detail of the largest frost crack present in the 1962 section at Teindland.



Climatic data presented by Dansgaard *et al.*[11] show that the period 28,000–12,000 b.p. was the most prolonged cold interval during the Devensian stage. The strong indications that the till-like material above the buried organic horizon at Teindland is a solifluction deposit and the probability that the frost cracks in the buried layer are contemporaneous with those developed in red till sections around the site suggest that the age of deposition of the local red till is pre-Devensian, and that Devensian sheet glaciation in this area is improbable.

I thank Mr J. Logan of the Macaulay Institute for the mechanical analyses in Table 1, Dr S. E. Durno of the Macaulay Institute for pollen analyses of the Bogincur farm buried peat and Mr J. Atterson of the Forestry Commission who took the photograph reproduced in Fig. 1.

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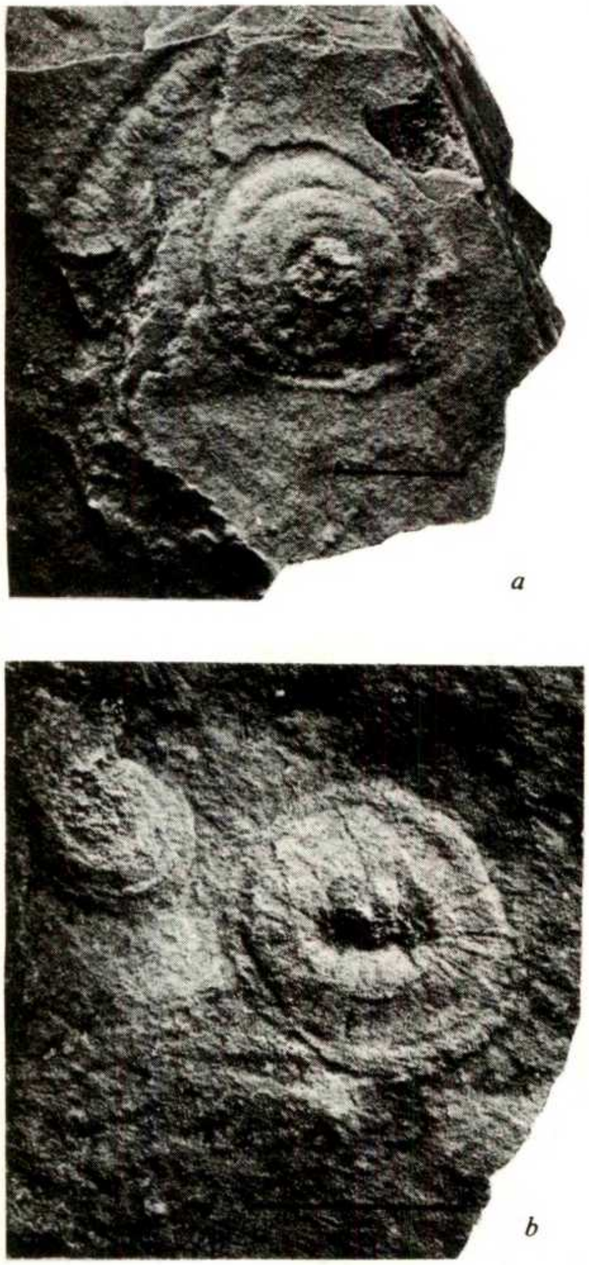
An Ediacara-type fauna from South Wales

DURING investigation of the geology of the area south of Carmarthen, South Wales, UK, an isolated small exposure of a finely inter-laminated mudstone and siltstone of possible volcanogenic origin was discovered. This lithology differs from those found hitherto in the Arenig and Old Red Sandstone rocks locally, and does not resemble any of the facies of the recently discovered Tremadoc rocks of the area[1]. Samples from this quarry were examined by K. J. Dorning of Sheffield University for microflora, but yielded none. Late last year a few badly preserved disk-shaped impressions, some 1 cm in diameter, were discovered. These were tentatively linked with the trace fossil *Astropolithon* by P. Crimes (Liverpool) and A. Seilacher (Tübingen), who both suggested that if the material proved to be *Astropolithon* an Early Cambrian age would be indicated.

Fig. 1 In each case, scale bar equals 10 mm. *a*, *Cyclomedusa* sp. *b*, *Medusinites* sp.



Further searches were made in the hope of finding material useful for dating. These have now yielded about 50 specimens including some better preserved material which is clearly of medusoid nature. A few of these specimens were subsequently examined by I. P. Crimes and by R. Jenkins (Adelaide), who both confirmed the medusoid nature of the fossils and suggested that they included a form occurring in the late Precambrian Ediacara fauna of South Australia.

Amongst the material so far collected, the genera *Cyclomedusa* Sprigg 1947 (ref. 2) and *Medusinites* Glaessner and Wade 1966 (ref. 3) have been identified. The less well preserved material includes a form resembling the genus *Tribrachidium* Glaessner 1959 (ref. 4). Others cannot at the moment be related to any previously described forms.

The fauna clearly carries elements characteristic of the Ediacara fauna. Late Precambrian soft-bodied organisms are known from various areas of the world[5] including Charnwood Forest, Leicestershire, UK[6]. In the Carmarthen region, however, until recently the earliest known rocks were of Early Ordovician (Arenig) age. The discovery of Tremadoc rocks there has led to a re-assessment of the age of the underlying rhyolites which are now presumed to be Precambrian. The rocks which have yielded the medusoids appear to rest on the rhyolites, although field relations in such a poorly exposed area are not clear.

No Cambrian rocks (if one excludes the Tremadoc) have hitherto been found in this area, and the possibility that the fauna is of Cambrian age cannot be excluded. It is felt, however, that the absence of any similar fauna in the Cambrian rocks of the St David's area (or elsewhere in Wales), argues against this, as does the apparently complete absence of metazoan skeletal material which would be most unusual for shallow water Cambrian sediments. Glaessner[5] has given reasons for assigning a late Precambrian age to the Ediacara fauna. On palaeontological as well as regional geological grounds a similar age is suggested for this fauna.

Details of the location of the exposure and a description of the fauna will be published after a full investigation of the site.

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Tachigalia versicolor is a suicidal neotropical tree

WITHIN a year of flowering in *Tachigalia versicolor* Standl. and Wms (Leguminosae-Caesalpinoideae) the leaves drop, the fruit is released and the tree dies. Reproduction occurs synchronously at intervals of several years in this large, much-branched, canopy tree species, but not all of the large individuals in the forest canopy flower and die at once. The species is known from the evergreen and semi-deciduous lowland forests of Panama, south-east Costa Rica and north-west Colombia. Except for a brief mention[1] of the Amazonian *T. myrmecophila*, I have found no previous reference to reproduction followed by death in *Tachigalia* (including *Sclerolobium*, an apparently congeneric taxon[2,3]), nor in any other large, branched, dicotyledonous tree. My preliminary observations in Peru suggest that this behaviour is characteristic of several of the other 56 species in this genus, most of which occur in the Amazon Basin.

For 10 yr, *T. versicolor* trees have been watched on Barro Colorado Island, Panama, a field station of the Smithsonian Tropical Research Institute in the centre of the Panama isthmus. Initially, nearly 40 individual trees were known and mapped though not tagged. In the past 3 yr a search has revealed 430 trees, and these have been mapped and marked with metal number tags.

From 1967 to 1969, no reproductive individuals were observed. But in 1970 at least 18 large trees flowered. All died within 1 yr, after release of their wind-dispersed fruits. No vegetative regrowth has been observed from the bases of trees or underground parts.

After 1970, no further flowering was seen for 4 yr. In 1974, eighty-five individuals were found in full flower on the island and all were dead by May 1975 (Fig. 1). This represents approximately one-fifth of the 430 known canopy trees. The non-reproductives completely overlap the range of sizes (height and diameter) of the reproductives (Fig. 2). Stem growth-rings are not distinct and the age of individuals at reproduction is not known.

Though the two flowering waves were distinct, the individuals flowering within a given year were not closely synchronised in the initiation of flowering. Some started flowering as early as January (the dry season) and others as late as July (the rainy season). Individual flowering lasts between 3 and 6 months. As a consequence, the flowering period for the whole population lasts almost a full year.

Two trees initiated flowering in 1975. One had its roots naturally grafted to those of an adjacent tree that had flowered in the previous year, and the second was a sickly individual with one branch remaining. No trees were observed to flower in 1976. There were a few partial exceptions to the rule of death following reproduction in 1974: three individuals produced flowers and fruits on only a few of their branches; those branches subsequently died, but the trees as a whole survived.

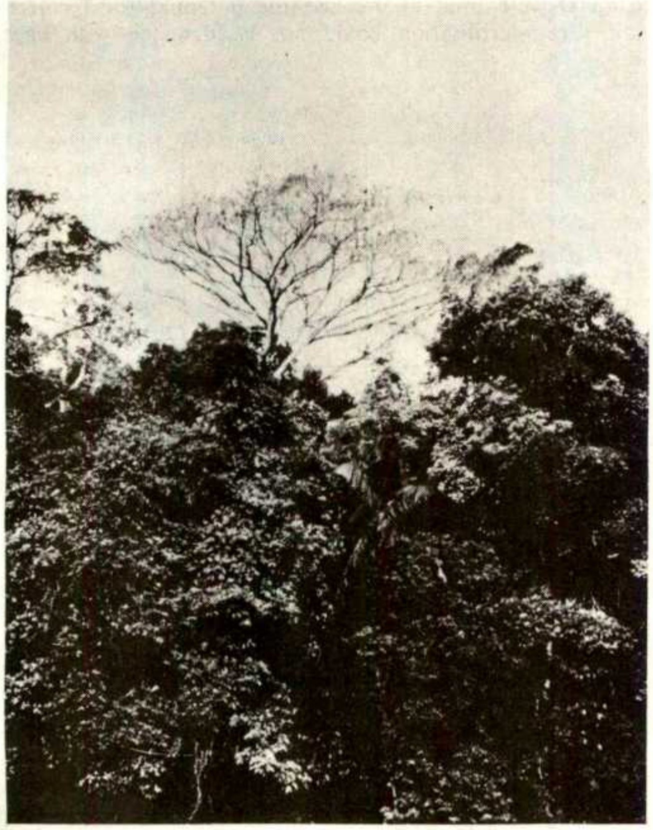

Fig. 1 View of dying emergent *Tachigalia versicolor* on Barro Colorado Island from Gatun Lake, Panama, February 1975. A few fruits remain on the estimated 7,000 terminal inflorescences. As flowering finishes, the leaves drop off, leaving the canopy crowded with 15-cm fruits that are green and leaf-like. These turn brown and are dispersed by wind in the January–April dry season, as the trunk starts to decay.

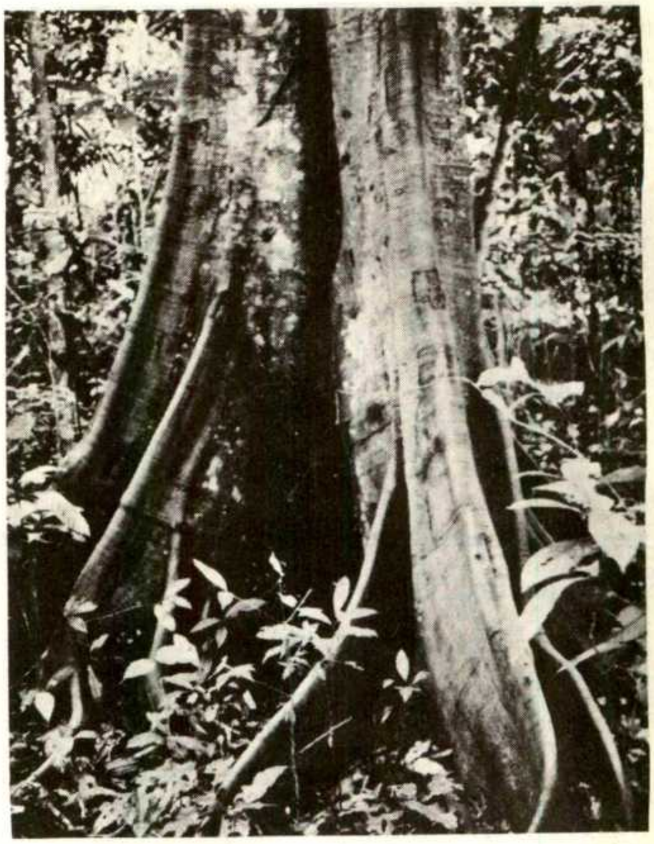

Fig. 2 Trunk of non-reproductive *Tachigalia versicolor* Leguminosae-Caesalpinoideae. Diameter at breast height was 116 cm and height was 35 m. Individuals of both reproductives and non-reproductives are commonly more than 150 cm and 40 m in height.

A suicidal act of reproduction might seem maladaptive for a species of huge trees. These trees do not seem to store nutrients and energy for the one reproductive event, for the quantities of flowers and fruits seem no greater than in comparably sized tree species with repeated reproduction. On the basis of initial observations, I propose that selection has favoured individuals which, by dying and falling, greatly increase the probabilities for their few progeny of survival to maturity in the forest canopy. The parent's death creates a more favourable environment for the development of its offspring, and occurs when the offspring are still abundant—having not yet succumbed to all the sources of juvenile mortality. Because most, if not all, tropical canopy species require an opening or 'light-gap' in the forest canopy for successful maturation[4,5], a treefall should strongly augment the opportunities for seedlings in the vicinity of the parent. As with other species that have large wind-dispersed propagules, the seeds and seedlings of *T. versicolor* are rarely found more than 100 m from the parent. Thus most seedlings (when not completely crushed) are likely to be exposed by the fallen parent, or by nearby treefalls resulting from the initial disruption of the forest canopy. *T. versicolor* saplings are often found growing in the openings directly created by the fall of an adult, or in adjacent openings. The rate of growth (or some other important parameter) of the trees I observed could be augmented by the amount of energy and nutrients they do not put into reproduction every year.

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Cross-fertilisation in fucoid seaweeds

EXPERIMENTAL studies on the extent of cross-fertilisation between eggs and sperm of various fucoid seaweeds are few, the results widely divergent and in no case have the experimental conditions been precisely defined. For example, in interspecific crosses between *Fucus serratus* eggs and *Fucus vesiculosus* sperm, values of 0%, 2% and 91% have been recorded (refs 1, 2 and 3 respectively). Hybridisation to a greater or lesser extent on the shore does, however, seem feasible especially since many species are cytologically identical[4]. Indeed, plants showing morphological features intermediate between parental types have been recorded[5,6]. While some authorities[7,8] consider that intermediate forms represent no more than ecotypes or phenotypic variants of parental type species, others[3] consider that these represent true hybrids, although cytogenetic evidence is lacking. We report here a re-appraisal of the specificity of cross-fertilisation between three fucoid seaweeds. The results show the presence of inherent interspecific and inter-generic barriers to fertilisation. But, interspecific hybridisation may be permitted following the breakdown of these barriers.

Fucoid eggs secrete a polysaccharide cell wall immediately after fertilisation[10,11]. Accordingly we have used the fluorescent brightener Calcofluor white ST (Cyanamid) to stain zygotes permitting a rapid and direct assessment of the level of fertilisation by ultraviolet microfluorescence (Reichert Diapan microscope fitted with exciter filters BG38 and UG1 and barrier filter GG13+W2B). It has been determined that zygotes which stain positively with Calcofluor subsequently show rhizoid development.

Fertile plants of *Fucus serratus, F. vesiculosus* and *Ascophyllum nodosum* were collected from several localities on the west coast of Anglesey, Wales during April 1977, and stored moist for up to 7 d. *Ascophyllum* was included in view of a report[9] that sperm of this plant can cross-fertilise eggs of *F. vesiculosus* to an extent of 4%. Dioecious species only were chosen for this study since it was considered imperative that any possibility of cross-contamination of gametes should be avoided.

Gametes were released[10] when required and crosses were carried out by incubating 5,000 eggs in concentrations of sperm up to 4,000 per egg, in a total volume of 2 ml for 20 min at 18 °C. Sperm were inactivated by adding 20 μl of 0.2% I_2 in 2% KI and the excess liquid removed from the eggs which had been allowed to settle. The eggs were washed once with seawater and then incubated for 5 min in a saturated solution of Calcofluor in seawater. After removing excess Calcofluor the eggs were washed in seawater once more, then percentage fertilisation was assessed by counting the number of eggs showing the characteristic fluorescence in a sample of 200 eggs. Control incubations omitted sperm.

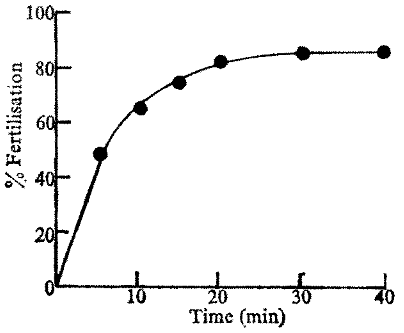

Fig. 1 Time-course of homologous fertilisation in *Fucus serratus.* 5,000 eggs were mixed with 200 sperm per egg.

Homologous fertilisation in the three seaweeds has similar characteristics with regard to the kinetics of fertilisation and fertilisation efficiency. Fertilisation can be detected within a few minutes of adding sperm but is essentially complete within 20 min (Fig. 1). Fertilisation efficiency is linear at low sperm concentrations, 50% of eggs being fertilised at relative sperm concentrations between 50 and 100 sperm per egg, saturation being approached between 250–500 sperm per egg (Fig. 2*a,e,i*). Efficiency of homologous fertilisations is independent of the stored age of either male or female plants up to 7 d in the experimental conditions used.

In heterologous fertilisations sperm to egg ratios of up to 4,000 were used to increase the potential for fertilisation. Cross-fertilisation could not be detected with eggs

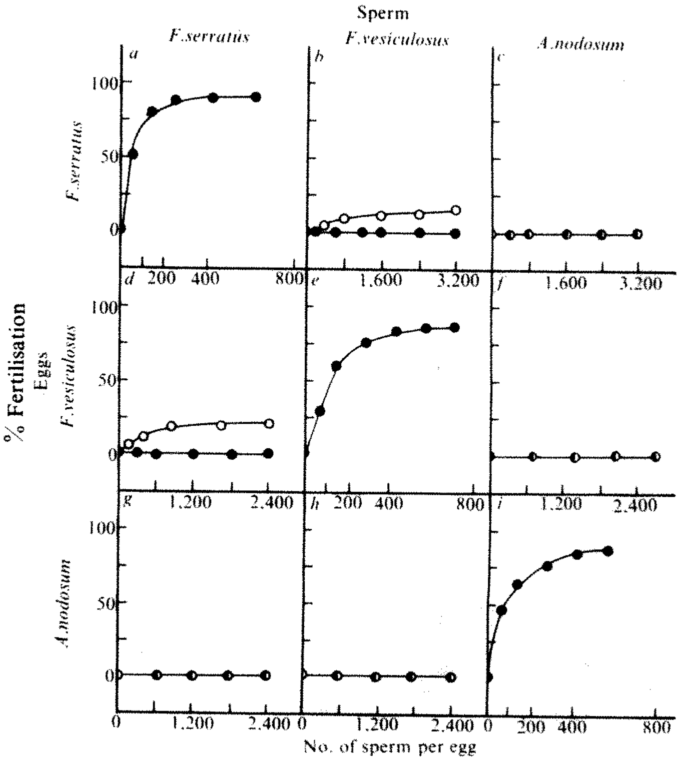

Fig. 2 Fertilisation efficiencies in homologous and heterologous fertilisations. 5,000 eggs were incubated with different concentrations of sperm. ●, Eggs from freshly-collected plants; ○, eggs from 4–5 d stored plants; ◐, eggs from freshly-collected or stored plants.

released from freshly collected plants in any of the six crosses shown in Fig. *2b–d,f–h*. But, in the case of *F. vesiculosus* eggs × *F. serratus* sperm, or the reciprocal cross, low levels of fertilisation were detected up to 20% with eggs freshly released from plants stored for 5–7 d (Fig. *2b,d*). Storage time of male plants was immaterial.

The inter-generic crosses between the two *Fucus* spp. and *Ascophyllum* were negative in all conditions of storage and sperm concentrations used. The occurrence of cross-fertilisation between *F. serratus* and *F. vesiculosus* was subsequently confirmed by the occurrence of segmented zygotes in samples incubated for 24 h.

Fucoid seaweeds show a wide range in form in response to environmental factors[12]. In addition, hybrids have been described and on the basis of ecological observations and hybridisation experiments it has been concluded that there are no inherent sterility barriers among *Fucus* species and that the barriers which prevent the merging of the different species into one polymorphic complex are ecological rather than physiological, related, for example, to non-coincidence of fruiting periods[3] and competition from parental forms[2]. The results of our investigation do not support this conclusion, since direct studies on fertilisation showed that there are inherent barriers to both inter-generic and inter-specific cross-fertilisation. But, the interspecific barriers seem to become less pronounced with age.

Gametes of the two *Fucus* species used in this study are continually differentiated and the older, more mature gametes are periodically released twice per day in the normal tidal cycle. Since stored plants were not subject to this tidal action the population of gametes experimentally released from stored plants will contain a relatively higher proportion of older eggs.

Specificity in seaweed fertilisation, as in sea-urchin[13] and mammalian[14] fertilisation is probably based on surface-localised complementary macromolecular recognition phenomena. It is tentatively suggested, therefore, that the partial breakdown of an inherent sterility barrier in the interspecific crosses is due to modification in the properties of the appropriate membrane receptors of the older egg cells. The molecular nature of these receptors is being investigated now. But, until more is known about the effects of external factors on the biochemistry of gamete development and the resulting implications on fertilisation, speculation on the ecological significance of the reduced specificity would be premature.

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Hedgehogs use toad venom in their own defence

I REPORT here that toxic secretions evolved in prey organisms such as toads (*Bufo*) as chemical anti-predator mechanisms are used by hedgehogs (Insectivora, Erinaceidae) to enhance their own mechanical anti-predator adaptations—the spines, modified hairs, of the back. The secretions are taken into the mouth and licked on to the spines. In spite of interest in chemical, mechanical and behavioural anti-predator adaptions of both invertebrates and vertebrates[1] there have been no reports of the behavioural utilisation of the anti-predator secretions of one species by another for its own defence. Toad skin is one of many irritating substances used by hedgehogs in self-anointing, and the presence of any of these, fresh or dried, on the spines would probably increase the pain or potential of infection to the would-be predator.

The survival value of spines is enhanced by the hedgehog rolling into a ball or 'boxing' when contacted or approached by a predator. Boxing consists of erecting the spines over the head and lunging into the approaching predator. Hedgehogs also display a curious behaviour pattern known as *Selbstbespeicheln* or self-annointing. Published reports[2–4] and my observations on 18 *Atelerix pruneri* and one *Hemiechinus auritus* agree that this behaviour involves frothing at the mouth then spreading the froth with the tongue on to the spines. Many possible functions of this behaviour have been considered and rejected[2].

Substances which naturally release self-annointing behaviour are either novel or noxious (irritating to mucosal membranes). Other common releasers are tobacco, soap and salamander skin secretions. Toad skin has often been mentioned as a releaser. Because toads (*Bufo*) occur throughout the natural range of hedgehogs, are commonly eaten by hedgehogs[5] and produce toxins in the granular glands of the skin it is possible that hedgehogs use *Bufo* skin toxin in their own defence.

Each time a toad (50+ times) with skin intact was contacted by adult or laboratory-reared juvenile hedgehogs they displayed self-annointing behaviour. When the following species of *Bufo* were offered to hedgehogs, the results were identical: *B. alvarius, B. americanus, B. boreas, B. marinus, B. quercicus, B. regularis, B. terrestris* and *B. woodhousei.* Toad skin, fresh (six trials) or dried (six trials), or excised toad paratoid glands (15 trials) always elicited self-annointing, but toads with the skin removed (six trials) were always eaten without self-annointing. Bullfrogs, *Rana catesbeiana,* and white mice (at least 30 each) were eaten without self-anointing. When hedgehogs attack toads they first bite and chew the paratoid glands (these contain the greatest concentration of skin toxins) then distribute the resultant frothy mixture of toad skin secretion and saliva over the spines (Fig. 1); the toad is then often eaten. In two cases *Hemiechinus* grasped the toad in its mouth and rubbed the skin over its spines (Fig. 2).

Observations of six *Atelerix* born and raised in captivity indicate that the self-anointing (Fig. 3) and other anti-predator behaviour are expressed before the eyes open at age 18–24 d. Long white spines are emergent within minutes after birth and a second stiffer set of dark spines emerges within 2–4 d. From 6 to 10 d after birth the young begin boxing behaviour, orienting the body towards any contact, and hissing. They can roll into a full ball by 2 weeks. Self-annointing is fully developed at 15–16 d (13-d-old juveniles do not exhibit this behaviour) in response to various stimuli such as sour milk, hands freshly washed with soap, or tobacco juice. These juveniles also licked substances from their mother's spines and applied them to their own spines.

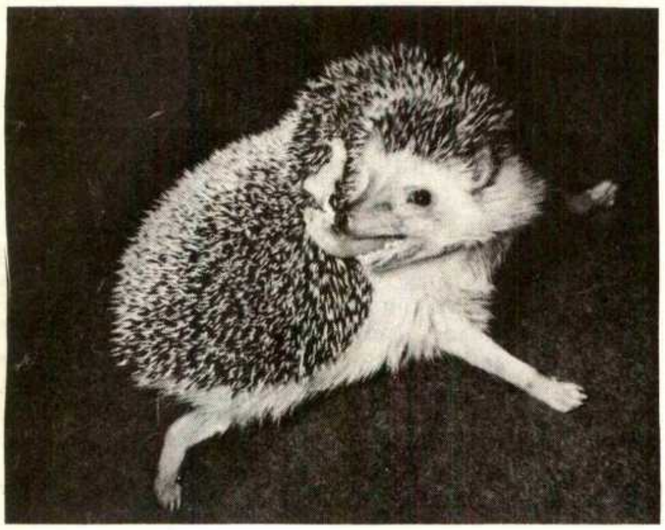


Fig. 1 Adult *Atelerix* self-annointing after chewing a toad (*Bufo*). The forth of saliva and toad skin secretion is being applied to the spines with the tongue.

To determine whether self-annointing with toad skin secretion would increase the pain inflicted by penetration of a hedgehog spine, spines were removed from an *Atelerix* and jabbed into the inner forearm of the author and six volunteers. Each subject jabbed himself with four spines in random order (only the author knew the order). The spines were treated as follows: (1) spines washed in alcohol; (2) spines washed then coated with hedgehog saliva; (3) spines washed then coated directly with *Bufo marinus* skin secretion; (4) spines freshly self-annointed with *Bufo marinus* skin secretion. In only one of seven cases did spines of groups (1) or (2) cause any reddening, and there was no pain or burning other than the mechanical pain of puncture. In group (3) six of seven subjects and in group (4) all subjects experienced immediate intense local burning. Splotchy red areas 8–46 mm ($\bar{x} = 24.6$ mm) developed around the puncture and reached their greatest development in 10–40 min ($\bar{x} = 18.1$ min). Burning lasted for 5–60 min and varied greatly in intensity. The reaction to two self-annointed spines dried for 1 week was the same as to freshly self-annointed spines.

Self-annointing behaviour is not only widespread among hedgehogs, having been reported in *Erinaceus, Aethechinus, Paraechinus, Hemiechinus* and *Atelerix*[2], but has also been described in the hedgehog tenrec *Echinops*[6], an insectivore of the family Tenrecidae convergent with the true hedgehogs in the presence of spines. Tenrecs, in contrast to hedgehogs, wipe the mixture of stimulating substance and saliva over the spines with the forepaws. In hedgehogs the mixture is always licked directly on to the spines, never on to the forepaws. These stereotyped behaviour patterns of both hedgehogs and tenrecs are released by chemical stimuli perceived by taste or smell, involve increased salivation, and include the spreading of this saliva–substance mixture on to the spines. The behavioural components of self-annointing in the two families of insectivores are quite different and were apparently developed independently[6]. The presence of these different behaviour patterns which both utilise saliva as a vehicle for the application of irritating substances on to spines suggests that these behaviours increase fitness. The fact that the anti-predator behaviour patterns including self-annointing are innate and fully developed in hedgehogs before leaving the nest further supports their importance.

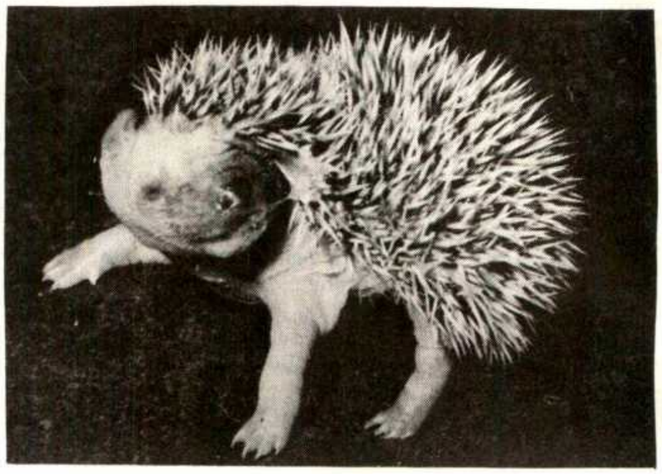
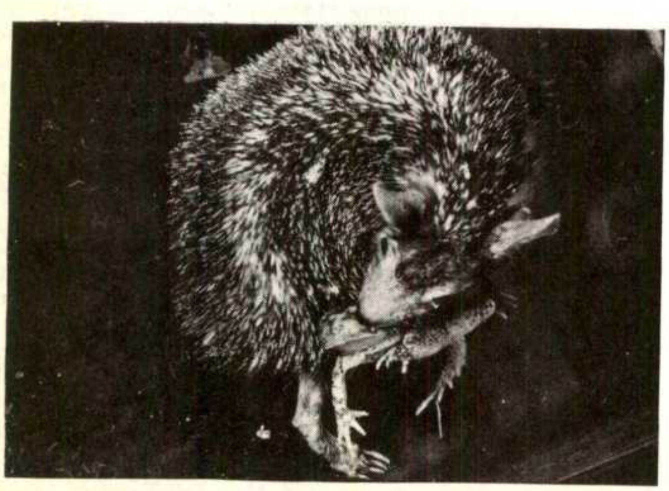


Fig. 3 Seventeen-day-old juvenile *Atelerix* frothing at the mouth and self-annointing after licking tobacco juice. The eyes have not yet opened.

Fig. 2 *Hemiechinus* rubbing *Bufo* against spines. Froth is visible on spines along the side and between the ears.



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Diets of early Miocene African hominoids

MOST reviewers of East African early Miocene apes have been impressed by their similarity, as an adaptive array, to living Cebidae. These apes are most frequently pictured as small to medium-sized cebid-like quadrupeds occupying arboreal[1-6], fruit- and leaf-eating[3,7,8], tropical rainforest[9] niches. It has also been suggested that the decline in the diversity of apes in middle to late Miocene times was triggered by ecological competition from rapidly radiating cercopithecids[9-11]. In this report

I examine the molar structure of early Miocene hominoids to see whether or not these species occupied a wide spectrum of fruit- and leaf-eating niches, similar to those of today's cercopithecids.

I have analysed 65 specimens of extant pongids and hylobatids from the Museè Royal de l'Afrique Centrale, the American Museum of Natural History and the US National Museum of Natural History. The sample includes 10 of the 11 recognised ape species[12]. The early Miocene fossil apes come from the collections of the Kenya National Museum and the British Museum of Natural History. Three species of New World monkeys were also examined. Twelve previously described dimensions[13] of unworn lower second molars were estimated: The mesiodistal M_2 length, used as an estimate of overall size; the heights of the metaconid and hypoconid; the lengths of shearing blades 1–8; and the summed surface areas of the trigonid and talonid basins. Cusp heights are interpreted as reflecting capacity for resistance to dental wear; shearing blade lengths and total basin areas reflect the relative importance of shearing, crushing and grinding in mastication. To eliminate the effects of allometry on these variables, I selected different-sized species with diets composed primarily of fruit (two *Pan* and five *Hylobates* species). For this group, 11 regression lines for the $\log_e$ of the molar dimensions (with $\log_e$ length as the independent variable) were fitted by the least squares method. The distance each species' molar dimensions falls above or below the *Pan-Hylobates* regression was expressed as a percentage of the expected dimensions for that tooth length[14]. The percentage values derived from each of the 11 regressions were used to produce a correlation matrix from which principal components were extracted. The resulting factor loadings (Table 1) were used to produce principal-component values for each specimen studied. The mean principal-component value for each species is shown in Fig. 1. Student's *t* tests were used to assess the significance of differences between the species means on each component axis. The factor loadings derived from this analysis also were used to produce principal-components values for specimens of living ceboids, Old World monkeys and fossil hominoids. The species means for these forms are shown in Fig. 1. In another principal components analysis, all living and fossil species were run together. The resultant factor loadings and principal-component values were extremely similar and are not reported.

Table 1 Factor loadings for the first two principal components of the analysis of *Z*-score corrected, % difference data of the lower second molars of 11 extant apes

Variable	Factor loadings Component 1 (50% of variance)	Component 2 (17.7% of variance)
M_2 hypoconid height	0.878	−0.034
M_2 metaconid height	0.596	0.339
M_2 shearing blade 1	0.363	0.484
2	−0.891	0.102
3	0.277	0.703
4	−0.674	0.500
5	0.767	0.438
6	0.587	−0.587
7n	−0.759	0.494
8	−0.780	−0.052
Total crushing area (9,10 n, x)	0.940	0.248

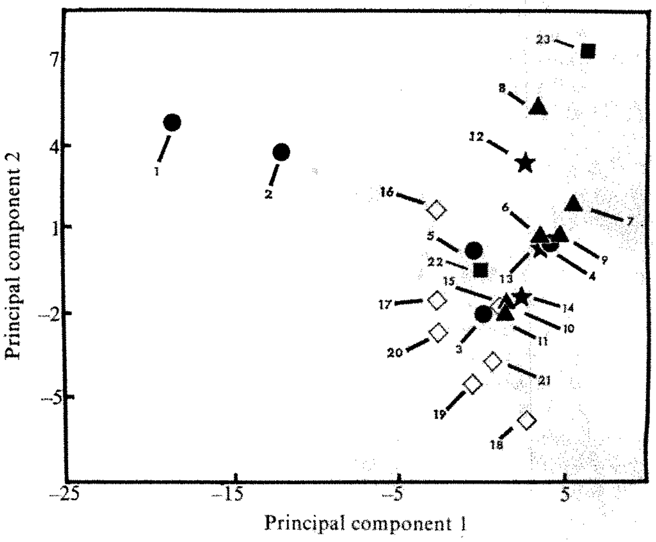

Fig. 1 Plot of principal components scores of primate M_2s along the first two principal components axes. Sample sizes are included in parentheses. Extant Pongidae (●): 1, *Gorilla gorilla beringei* (8); 2, *Gorilla gorilla gorilla* (6); 3, *Pan troglodytes* (10); 4, *Pan paniscus* (11); 5, *Pongo pygmaeus* (5). Extant Hylobatidae (▲): 6, *Hylobates lar* (5); 7, *Hylobates hoolock* (2); 8, *Symphalangus syndactylus* (3); 9, *Hylobates moloch* (5); 10, *Hylobates klossi* (5); 11, *H. agilis*. Cebidae (★): 12, *Aloutta seniculus* (5); 13, *Ateles belzelbuth* (5); 14, *Cebus capucinus* (5). Extinct Hominoidea (◇): 15-17 Songhor; 15, '*Limnopithecus*' *legetet* (3); 16, *Dryopithecus gordoni* (6), 17, *Dryopithecus maor* (3). 18–21, Rusinga Island; 18, '*Limnopithecus*' *legetet* (2); 19, *Dryopithecus africanus* (5); 20, *Dryopithecus nyanzae* (7); 21, '*L.*' *macinnesi* (8). Cercopithecidae (■): 22, *Cercocebus albigena* (6); 23, *Colobus badius* (6).

The primarily folivorous species, *Gorilla*[15] and *Symphalangus*[16] are clearly separated morphologically from other, more frugivorous, pongid and hylobatid species[17–9] on the first and second principal component axes which account for 67.7% of the total variance of the system (Fig. 1). The first principal component separates *Gorilla gorilla* from all other species by a combination of high cusps, enlarged crushing basins and elongate molar crests 5 and 6 (Table 1). But, the first principal component does not separate folivorous from frugivorous hylobatids. The second principal component separates folivorous from frugivorous species. The former have relatively high metaconids and a great overall amount of shearing and crushing (Table 1). The mean second principal-component values of *Gorilla gorilla* subspecies and *Symphalangus syndactylus* are significantly larger (at the 95% confidence level) than those of all other pongid and hylobatid species tested. This result conforms with the diet–molar morphology correlation seen in other mammalian groups. Folivorous species of Old World monkeys and phalangeroid marsupials also have better developed shearing blades than frugivorous species[20]. Leaves, bark, buds and other folivorous materials tend to contain large amounts of structural carbohydrates (cellulose, hemicellulose) which require thorough trituration facilitated by well developed molar structures, to enhance digestibility[20]. In contrast, fruits tend to be lower in structural carbohydrates and higher in soluble carbohydrates which are highly digestible even without full trituration[21].

Before using this principal components analysis as a model for comparisons of Miocene species, its reliability as a predictor of dietary habits was tested for representative folivorous and frugivorous Old and New World monkeys. To do this, *Z*-standardised percentage scores were tabulated from the *Pan–Hylobates* regressions for the highly frugivorous New World species *Ateles* and *Cebus*[22] and the folivore *Alouatta*[22,23]. These species' values are interpolated into Fig. 1. *Ateles* and *Cebus* fall with the frugivorous hylobatids and pongids on the second principal component and have significantly smaller (at the 95% confidence level) second-component values than do the folivorous hominoids. The second principal-component value for *Alouatta* is significantly larger than all frugivorous ceboids and hominoids analysed, and is not significantly different from the more folivorous *Symphalangus* or *Gorilla* species. Apparently, the morphological distinctions between fruit- and leaf-eating cebids are analogous to those seen between fruit- and leaf-eating hominoids. Also interpolated into Fig. 1 are the principal-component values for two species of cercopithecoid monkey, *Cercocebus albigena* and *Colobus badius*. The former is primarily a fruit eater whereas the latter eats primarily leaves. *Cercocebus* falls with primarily fruit-eating hominoids and cebids, whereas *Colobus* falls with the leaf-eating species of these groups. It may be concluded from the above that second lower molar morphology can be used to reliably separate extant fruit-eating from leaf-eating Higher Primates.

Lower second molars of fossil apes from two East African Miocene sites, Songhor and Rusinga Island, spanning the time period of 18–20 myr BP (ref. 24) were measured. The material included specimens of six of the seven species known from these sites[9]: *Dryopithecus gordoni*, *D. major*, *D. nyanzae*, *D. africanus*, '*Limnopithecus*' *macinnesi* and '*L.*' *legetet*. The lower dentition of *D. vancouveringi* is not yet known (identified). As with the Old and New World monkeys reported above, *Z*-standardised percentage scores were calculated from the *Pan–Hylobates* regressions, principal-component values were calculated for individual specimens and the species, mean was determined for each species grouped by locality. The species' values are plotted in Fig. 1. The Songhor specimens of *D. major*, and '*L.*' *legetet* fall with living frugivorous hylobatids on the second principal component, and are very close to *Pan troglodytes* as well. Both the fossil species have significantly smaller second-component values than extant folivorous species at the 95% confidence level. A frugivorous diet for these species is indicated. *Dryopithecus gordoni* falls between the ranges of living frugivores and folivores on the second principal component axis. This species is not significantly different from either *Gorilla gorilla gorilla* or from several of the species of *Hylobates*. Rusinga Island specimens of *D. nyanzae*, *D. Africanus*, '*L.*' *legetet* and '*L.*' *macinnesi* fall with frugivorous extant hominoids and have significantly smaller second-component values than extant folivorous species. Points 15 and 18 in Fig. 1 representing '*L.*' *legetet* from two different sites are not significantly different from one another at the 95% confidence level. This analysis strongly indicates that the known early Miocene hominoids (with the possible exception of *D. gordoni*) probably had diets consisting largely of fruit. In this respect they show less dietary diversity than the pongids of hylobatids of today, since members of both those groups have folivorous representatives. Judging from the molar morphology *D. major* has no apparent dietary affinity with *Gorilla*, contrary to previous suggestions[8].

Early Miocene apes apparently were quite similar, adaptively, to some extant cercopithecines and a case can be made that monkeys replaced apes in middle to late Miocene times. Early apes have been collected at sites which sample tropical rain-forest environments[9] where cercopithecines are abundant today, in terms of species numbers. Early apes had far greater species diversity than the apes of today and in this respect they mirror the diversity of living forest-dwelling cercopithecines[9]. Dental dimensions suggest that early apes and living forest dwelling cercopithecines have a similar body size range (2–40 kg). Most of the early Miocene apes are similar to extant cercopithecines in being arboreal quadrupeds[1-6] with some of the larger forms being at least partially terrestrial[7]. Finally, early Miocene apes were primarily frugivorous, as is the case with many arboreal cercopithecines of today.

But these similarities should not be taken as demonstrating an identity in the adaptive zones of early Miocene apes and extant forest monkeys. There is no evidence that any early Miocene hominoids fed on seasonally high proportions of leaves, bark, buds, or grasses, as do some cercopithecines today. There were no colobine vicars among the early Miocene hominoids. Thus, in addition to displacing early hominoids, the cercopithecid adaptive radiation may also have come at the expense of other arboreal and semiterrestrial species. Possibly the African colobine leaf-eating radiation displaced some hyracoids, anomalurid rodents or arboreal reptiles, some of which eat bark and leaves[25,26].

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Further study of an auditory illusion

DEUTSCH[1] reported an interesting auditory illusion when different tones are presented to the two ears. She used chords alternating every 250 ms between 400 Hz to the left ear, 800 Hz to the right and 800 Hz to the left, 400 Hz to the right. Subjects never heard the correct pattern; they perceived most often a low tone in one ear alternating with a high tone in the other. Right-handed subjects tended to hear the high tone in the right ear and the low tone in the left ear, but left-handed subjects showed no such tendency. Deutsch and Roll later found that right-handed subjects tended to report the sequence delivered to the right ear rather than that to the left, but the tone reported tended to be localised in the ear receiving the higher frequency[2]. They concluded that there were separate mechanisms for perceiving location and pitch, and that the right ear preference for pitch was related to cerebral dominance. Efron and Yund[3,4] reported a related phenomenon using a sequence of two pairs of tones : 1,500 Hz to the right ear, 1,900 Hz to the left followed by 1,900 Hz to the right ear, 1,500 Hz to the left. Again most subjects heard a single note each time. Efron and Yund systematically varied the relative intensity of the two tones and found that the location of the perceived sound depended critically on this variation, always being heard towards the side receiving the more intense sound. If the two intensities were exactly balanced for an individual, then the perceived sound was subjectively located in the midline. The subjective pitch of the sound heard was a separate function from its location, and frequently the pitch of the less intense sound was heard, but localised in the ear receiving the more intense sound. We have used Deutsch's frequencies of 400 Hz and 800 Hz, and also 457 Hz and 857 Hz. We presented two consecutive pairs of tones, systematically varying the intensity, and confirmed Efron and Yund's results. Using equal intensities we obtained different results from Deutsch.

The tones lasted 250 ms, with a rise and fall time of about 10 ms, and were presented at an intensity of 65 decibels 2×10^{-5} N m^{-2} through TDH-39 earphones.

Twenty-four right-handed subjects, without extensive musical training, were tested with an audiometer at the two frequencies used and any small difference in acuity was approximately balanced by adjusting the intensity of the tones. Subjects were first given each tone separately as standards of high and low, and then were allowed to listen to the pair of chords as often as they wished by pushing a button to obtain a presentation. For each pair of chords they were asked to indicate on a response sheet exactly what they heard in terms of pitch and location. Each subject received eight stimulus configurations; on two of these the tone of higher frequency was attenuated by 10 dB and on another two the lower tone was attenuated by 10 dB.

A second experiment on another 24 subjects was identical, except that frequencies of 457 Hz and 857 Hz were used, to see if there was any difference with tones lacking a simple harmonic relation.

The results relating to the relative intensity of the two tones are clear. In both experiments, when one of the tones was attenuated by 10 dB, then if only one tone was perceived it was located in the ear receiving the higher intensity on 85% of cases. The pitch of the tone heard was not necessarily that of the more intense tone. This confirms the results of Efron and Yund, that the location of the perceived sound depends on the relative effective intensity of the tones for each individual subject. It also suggests that Deutsch's finding that the high tone tends to be localised in the right ear and the low tone in the left may be due to small intensity differences, or to differences in acuity between the ears of individual subjects.

What subjects hear, as opposed to where they hear it, is a more complex function. Table 1 shows a summary of the results when the two tones are of approximately equal intensity. For each chord (High-L, Low-R or High-R, Low-L) is shown the number of times it was heard as a chord, or single high, low or medium note, irrespective of location. Each of the twenty-four subjects made four observations of each chord. Order effects were not significant.

Table 1 Number of times each chord was heard as a chord, or single high, low or medium pitched note

Stimulus frequencies	400 Hz/800 Hz		457 Hz/857 Hz	
Stimulus chord	High–L Low–R	High–R Low–L	High–L Low–R	High–R Low–L
Heard as:				
Chord	0	0	30	33
High	42	40	40	27
Medium	22	28	9	3
Low	32	28	17	33

L, left; R, right.

The perception of a single note is very clear using frequencies of 400 Hz and 800 Hz; a single note was heard on all occasions. With frequencies of 457 Hz and 857 Hz a single note was reported about 66% of the time—but nearly half the subjects heard a chord on some occasion. The illusion seems very strong if the notes have a simple harmonic relation and less strong, but still common, if they do not.

No overall pattern emerges from the detail of what is heard when only one note is reported, but Deutsch's result that the right ear determines what is heard is not confirmed. Whether the notes have a simple harmonic relationship or not, the stimulus configuration does not significantly influence the pitch of the reported note. The function determining what is heard is complex, and shows considerable individual differences. Individual subjects tend to be fairly consistent in their preferences.

The illusion falls into the class of 'curious binaural phenomena' reviewed by Tobias[5], who concluded that 'any similarity between two sounds may lead to their fusion, if the similar factors fall below 1,500 Hz and come close to coinciding in time'.

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Racial differences in the frequency of Q and C chromosomal heteromorphisms

THE biological and clinical implications of human chromosome heteromorphisms are poorly understood. These heteromorphisms are limited to certain groups, and are heritable[1]. Consistent variability between people, in both size and staining properties, is observed. This may reflect either structural or biochemical variations. Before chromosome banding procedures, the length of the Y was known to vary from person to person and from one ethnic group to another[2]. Some autosomal regions were also found to differ in various racial groups[3,4]. Using Q- and C-banding procedures, we have regions scored were (1) on the long arms (*qh*) of chromosomes 1, 9 and 16; (2) on the short arms (*p*) of the acrocentric autosomes (13–15 and 21–22); and (3) on the centromere (*c*) of all other chromosomes. The results indicate significant differences between the samples of black and white children.

Chromosome studies of peripheral blood lymphocytes were originally done on 4,342, 7–8-yr-old, children from the Collaborative Perinatal Study through the collaboration of cytogenetic laboratories in five cities. The culture and harvest methods are described elsewhere[5]. Most of the data presented here, however, were obtained from a subsample of 415 children. This was derived, using random numbers, from the larger study group and designed to give equal numbers of children of each race above and below an IQ of 85 (ref. 6). Slides from these children were sent to the coordinating centre for Q-banding. The same slides from 200 of the 415 children were also successfully C-banded. In all cases, 5–10 cells were photographed and analysed; two karyotypes were prepared. If C-banding was done, two dual karyotypes with Q- and C-banded chromosomes were prepared from the same cells. It is important to note that the C-banding of all chromosomes could be studied because each chromosome was preidentified by Q-banding. Without this sequential staining, only 1, 9, 16 and Y can be studied.

Heteromorphisms were scored according to predetermined criteria without knowledge of race, IQ or other parameters. For Q-banding, the five intensity levels established in 1971 at the Paris Conference were used[7]. The levels are brilliant, intense, medium, pale and negative; for convenience, they are referred to as levels 5–1, respectively. For C-band heteromorphisms, the karyotype was arranged by decreasing size of the C-band region from largest to smallest[8]. This permitted comparison of C-band regions on homologous and nonhomologous chromosomes having similar sized heteromorphisms. Classification of both Q- and C-band heteromorphisms was done by two independent observers, whose scores were virtually identical.

There were 190 black and 194 white children: 98 of the black and 52 of the white children had an IQ less than 85 and the remaining children (92 black and 142 white) had an IQ of 85 or above.

Before scoring the heteromorphisms, each set of slides was scored for quality of culture and quality of banding. There were no significant differences in these distributions between races or between IQ groups. In all groups, the number of heteromorphisms detected directly reflected the quality of the culture. On the average 3.3 Q- and 3.9 C-band heteromorphisms per child were found.

The frequencies of Q-band heteromorphisms in the two racial groups are given in Table 1. An average of seven heteromorphisms was present in black children and an average of five in white. Where there were significant racial Q-band differences, blacks tended to brilliant or intense heteromorphisms on chromosomes 3, 13 and 22. Chromosome 4 was the single exception. Even when the difference was not statistically significant, the frequency of level 4 and 5 heteromorphism tended to be greater in blacks. We conclude that bright Q-band heteromorphisms are generally more common in blacks.

The centromere (C-band) heteromorphisms were studied in 95 white and 97 black children. These were much less frequent than the prominent Q-band heteromorphisms or *qh* heteromorphisms in 1, 9 and 16. Therefore, data for all chromosomes were pooled. In the white children, the number with small ($N = 44$) and large ($N = 44$) C-band regions was equal, whereas 50 black children had small and 78 had large heteromorphic regions. Overall, larger than usual C-band regions were clearly more common in blacks ($P < 0.005$). Most of this difference was due to chromosomes 4, 18 and 19. No individuals in the white sample had prominent, large centromere heteromorphic regions for chromosomes 4, 18 and 19, but 7–9% of the black children had large centromere regions for these chromosomes. Five white children had a large centromeric C region in an X chromosome in contrast to only one black child.

Table 1 Frequencies of Q-banding heteromorphisms in white and black ethnic groups

Chromosome region	Level (s)	White ($N = 205$) *N*	White ($N = 205$) Prop.*	Black ($N = 210$) *N*	Black ($N = 210$) Prop.*	*P* value
3*c*	4	57	0.28	89	$\underline{0.42}$	< 0.01
	5	119	0.58	119	0.57	ns†
4*c*	4,5	42	$\underline{0.20}$	20	0.09	< 0.01
13*p*	4	87	0.42	113	$\underline{0.54}$	< 0.01
	5	22	0.11	70	$\underline{0.33}$	< 0.001
13*s*	4,5	9	0.04	20	$\underline{0.10}$	< 0.01
14*s*	4,5	23	0.11	27	0.13	ns
15*p*	4,5	2	0.01	6	0.03	ns
15*s*	4,5	21	0.10	21	0.10	ns
21*p*	4,5	1	0.005	4	0.02	ns
21*s*	4,5	16	0.08	28	0.13	ns
22*p*	4,5	4	0.02	8	0.04	ns
22*s*	4,5	12	0.06	29	$\underline{0.14}$	< 0.01

*Proportion of people, either heterozygous or homozygous. Significantly higher frequencies are underlined.

†ns, not significant χ^2 value.

The prominent C-band regions on chromosomes 1, 9 and 16 were also studied. These heterochromatic (*h*) regions are usually on the long (*q*) arm of these chromosomes just below the centromere and are termed *qh* regions. There were no obvious racial differences in either extremely small or large *h* regions for chromosomes 1, 9 or 16. Because of greater complexity of these data, they were analysed on a per chromosome basis, rather than a per person basis and will be reported separately by us. Occasionally, however, they seem to be inverted and to be partially or wholly on the short arm of these chromosomes. Although there is no information which permits us to determine whether these are true inversions or whether they represent unusually placed centromeres, the term 'inversion' has been used for a decade and remains reasonably descriptive. Partial inversions were scored when there was a dumbell-shaped C-region, with the centromeric constriction near the centre of the dark-staining C-band regions. Complete inversions were those in which all of the C-band positive material was found on the short arm. These heteromorphisms were analysed in the overall sample of 4,342 children since they can be reasonably well detected in G-banded and unbanded cells (Table 2).

Table 2 Frequencies of 1 and 9 *q* inversions* in white and black ethnic groups in the overall sample

Inversions	White ($N = 3084$) *N*	White ($N = 3084$) %	Black ($N = 1780$) *N*	Black ($N = 1780$) %	*P* value
1 *h* partial	14	0.45	1	0.06	< 0.05
9 *h* partial	17	0.55	8	0.45	ns
9 *h* complete	4	0.13	19	1.07	< 0.001

*'Partial inversions' were defined as those in which the centromere was in the middle of the *h* region and 'complete inversions' of chromosome 9 as those in which there was a metacentric 9 with the entire *h* region being in the short arm.

Complete inversion of the 9*qh* region was much (six times) more common in blacks, as had been reported before banding[4]. Conversely, partial inversions of chromosome 1 were much more common (eight times) in whites. No complete inversions of chromosome 1 and no inversions, even partial, of the C-band region on chromosome 16 were observed in our sample, suggesting that these inversions are uncommon in both racial groups.

Previously published population surveys using banding techniques have dealt solely with white populations[8–13]. Only in the present survey, therefore, can the possibility of racial differences be studied. There was clearly a significant black/white difference in the frequency of Q- and C-band chromosome heteromorphisms, with blacks generally having more prominent heteromorphisms than whites. Presumably this reflects a greater degree of redundancy of certain classes of human satellite DNA in these regions. Studies with annealing techniques have not yet been carried out in individuals of different racial or ethnic backgrounds. As with ethnic differences in blood groups, enzyme types and other single gene markers, the evolutionary significance of these frequency differences is purely conjectural. They are clearly of similar genetic and anthropological interest, however, and may be of value in planning linkage and population research.

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Androgenetic origin of hydatidiform mole

CLASSIC hydatidiform mole is the product of an abnormal pregnancy with grossly swollen chorionic villi, but without an embryo, cord or amniotic membrane. Histologically, the villi are characterised by advanced hyperplasia and anaplasia of the trophoblast, oedema of the stroma and the absence of foetal capillaries[1]. A malignant change to invasive mole or choriocarcinoma is frequent. The karyotype of the mole is predominantly, if not exclusively, 46,XX. We have made a cytogenetic study of the origin of hydatidiform mole and found that it receives only paternal chromosomes and is therefore androgenetic in origin.

Karyotyping was done on 20 moles: in 13 cases on uncultured villi, in three cases on cultured villi and in the other four cases on both cultured and uncultured villi. Ten moles were of Caucasian origin and ten were of Japanese origin. All had a 46,XX karyotype.

The mode of inheritance of molar chromosomes was studied using fluorescence Q and R-band polymorphisms as markers, as described before[2]. Seven moles, two Spanish and five Japanese in origin, and the parents were studied (Table 1). Chromosome slide preparations were made from both cultured and uncultured villi of molar specimens, and from short term lymphocyte cultures of the parents. Seven pairs of chromosomes (nos 3, 4, 13, 14, 15, 21 and 22) from each of the seven moles were scored for fluorescence banding polymorphisms (Table 1). In all of the 49 pairs scored, homologous chromosomes were homozygous for banding polymorphisms (Fig. 1). In 26 pairs of chromosomes, neither of the maternal homologues was transmitted to the mole. In 20 of the 26 pairs, a paternal chromosome could be shown to be inherited in duplicate by the mole (Fig. 2). Comparison of the paternal and molar chromosomes revealed that in 30 pairs the paternal homologues were heterozygous for banding polymorphisms, while those of the moles were homozygous (Table 2).

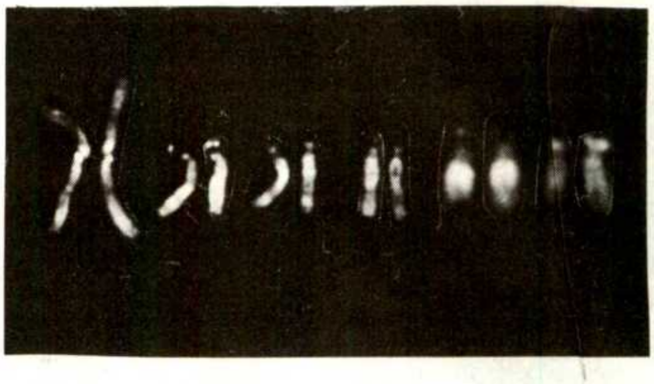

Fig. 1 Q-banded direct chromosome preparation from case 2. All the homologous chromosomes are homozygous for banding polymorphisms.

Stromal cells in villi were studied for the X chromatin in 47 moles, 18 Caucasian and 29 Japanese in origin. All but three were X-chromatin positive, varying in frequency between 73% and 85.5%. Of the three X-chromatin negative moles, two were Y-chromatin negative, while the remaining one was Y-chromatin positive in 54% of its stromal cells. Necrotic changes could be excluded as a cause for the latter findings. Karyotyping was not successful in the three X-chromatin negative moles.

A total of 35 couples each with a molar pregnancy were karyotyped, and two of the women had a balanced reciprocal translocation: t(7;8)(q22;p21) and t(3;22)(p11;p11). The mole from the first translocation carrier woman revealed a 46,XX karyotype, while that from the latter failed to yield a karyotype.

Our results indicate that moles are androgenetic in origin. Androgenesis is the development of an ovum under the influence of a spermatozoan nucleus, the egg nucleus being either absent or inactivated[3]. Androgenetic 'zygoids' are usually haploid. Diploid androgenesis, as in these moles, involves a male chromosome complement that has become diploid through fertilisation by two sperm (dispermy), by a diploid sperm or by a haploid sperm followed by duplication of its chromosomes. In the case of dispermy, however, there would be an equal number of heterozygous and homozygous chromosome pairs. The fact that all 30 informative chromosome pairs in the moles studied were homozygous essentially rules out this possibility. The chromosome findings are compatible with either fertilisation by a diploid sperm due to nondivision at second meiotic division or fertilisation by a haploid sperm followed by duplication of its chromosomes and nondivision at first 'blastomere' mitosis. These two possibilities are indistinguishable by the method we used. Both these events would produce a diploid zygoid containing either XX or YY sex chromosomes. Because

Table 1 Mode of inheritance of polymorphic chromosomes in hydatidiform moles

Case	Ethnic group	Chromosome preparation	Karyotype	Mode of inheritance in chromosomes nos 3	4	13	14	15	21	22	Total no. P	patII
(1)	Spanish	Direct	46,XX	patII			patII	pat	patII		4	3
(2)	Spanish	Direct Cultured	46,XX						patII	patII	2	2
(3)	Japanese	Direct Cultured	46,XX			pat	patII		patII	patII	4	3
(4)	Japanese	Direct Cultured	46,XX	pat		patII	patII	patII			4	3
(5)	Japanese	Cultured	46,XX		patII	patII	patII	pat	patII		5	4
(6)	Japanese	Cultured	46,XX			patII	patII	patII			3	3
(7)	Japanese	Direct Cultured	46,XX			pat		patII	pat	patII	4	2

Direct, direct preparation. Karyotyping was done without intervening culture period, in order to avoid possible outgrowth of maternal decidual cells. pat, Paternal in origin, ascertained through the exclusion of inheritance of maternal homologues. patII, Paternal second meiosis, ascertained through the transmission of a paternal chromosome in duplicate to the mole.

Table 2 Comparison of fluorescence-banding polymorphisms in paternal and molar chromosomes

Case	Tissue	Polymorphisms involving chromosomes numbers 3	4	13	14	15	21	22	Total no. Heterozygous	Homozygous
(1)	P	ab			ab		ab	ab	4	
	HM	aa			aa		aa	aa		4
(2)	P	ab					ab	ab	3	
	HM	aa					aa	aa		3
(3)	P			ab	ab	ab	ab	ab	5	
	HM			aa	aa	aa	aa	aa		5
(4)	P			ab	ab	ab	ab	ab	5	
	HM			aa	aa	aa	aa	aa		5
(5)	P	ab	ab	ab	ab		ab		5	
	HM	aa	aa	aa	aa		aa			5
(6)	P	ab		ab	ab	ab		ab	5	
	HM	aa		aa	aa	aa		aa		5
(7)	P				ab	ab		ab	3	
	HM				aa	aa		aa		3
Total no.		4	1	4	6	4	5	6	30	30

P, paternal; HM, hydatidiform mole; a and b do not represent two specific phenotypes, they are merely symbols.

a cell must have at least one X chromosome to survive, presumably only the XX 'zygoids' could survive to become moles.

The X-chromatin negative moles in our study may have had two X chromosomes with neither X inactivated. Alternatively, they may have arisen through a mechanism different from that in the seven moles of which the chromosome source has been studied. Abnormal first meiotic division in the male, for example, would result in XY diploid sperm, which in turn could produce XY moles. The X-chromatin negative, Y-chromatin positive mole in this study may have arisen by this mechanism.

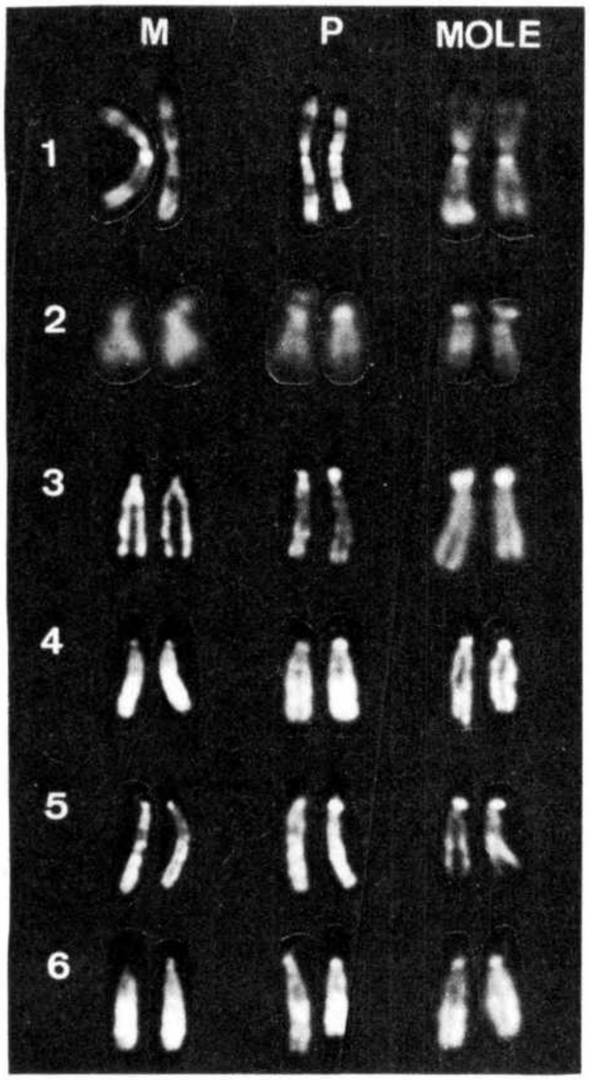

Fig. 2 Chromosomal polymorphisms demonstrating the differences between the maternal (M), paternal (P) and molar (mole) chromosomes. Cases 1, 2, 4, 5 and 6: Q-banding. Case 3: R-banding. Case 1: no. 3. Case 2: no. 22. Case 3: no. 14. Cases 4–6: no. 13. In each case, a paternal chromosome (right) was transmitted in duplicate to the mole, while neither of the maternal homologues was inherited by the mole.

The finding of two translocation carrier women among the 35 couples with molar pregnancies is interesting. Their frequency of 6% among the women studied is higher than that of 0.3% in the general adult population[4]. The presence of translocation chromosomes may interfere with female meiosis and encourage the production of ova with absent or inactivated nuclei.

The high malignancy rate in hydatidiform mole could be explained by a recessive mutation of a gene (or genes) which controls cell growth. Because the homologous chromosomes in the mole are genetically homozygous except for the genes exchanged through crossover, they are also homozygous for the mutation and thus may escape from normal growth control.

Wide geographic variations have been known in the frequency of moles, which are more frequent in Asian countries[5] as well as Latin American countries of the tropical zone[6] than European and North American countries. The results reported here indicate that the Asian disease is the same as that found among the Caucasians. This finding, however, fails to explain why its incidence is higher among Orientals. Study of the frequency of diploid sperm among Oriental males and that of defective ova among Oriental females could shed light on this question.

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Genetic model for observed distributions of proportions of haemoglobin in sickle-cell trait

IN individuals with sickle-cell trait, mature erythrocytes contain less haemoglobin (Hb) S than HbA. Although variations in the proportions of HbS and HbA have been recognised since quantification of the Hb fractions first became possible, the mechanisms responsible are incompletely understood. Nutritional deficiencies[1,2] and other non-genetic conditions[3,4] affecting globin synthesis may alter these proportions in individual heterozygotes but are unlikely to explain the variations found in population surveys. A simple genetic model which can account for the observed distributions of the proportions of HbS in sickle cell trait is presented here.

For most populations, the observed proportions of HbS in heterozygotes may be grouped into three phenotypic classes with mean values of 25% (range 20–29%), 33% (range 30–36%), and 40% (range 37–50%). The model assumes that these values are determined by two types of genetic units (explained below), G_1 and G_2, such that the phenotypes with 25, 33 and 40% of HbS correspond to the G_1G_1, G_1G_2 and G_2G_2 genotypes. If the respective frequencies of G_1 and G_2 are p_1 and p_2 then for a population in Hardy–Weinberg equilibrium, the genotype frequencies are given by the binomial expansion $(p_1+p_2)^2$ as shown in Table 1. Given the number of individuals observed in each phenotypic class, the gene frequencies p_1 and p_2, and the number of individuals expected in each class may be calculated[5]. The goodness of fit of these expected frequencies with observed phenotype frequencies may be evaluated with the χ^2 test[5].

Table 1 Phenotypes and genotypes: model with two genetic units (G_1 and G_2)

Phenotype (% HbS) Mean	Range	Model genotype	Frequencies
25	(20–29)	G_1G_1	p_1^2
33	(30–36)	G_1G_2	$2p_1p_2$
40	(37–50)	G_2G_2	p_2^2

Ten studies (refs 6–14 and G. Brittenham *et al.*, unpublished) have described the distributions of the proportions of HbS in 13 populations. Table 2 displays the observed and expected numbers of individuals in each of the phenotypic classes, the calculated gene frequencies for G_1 and G_2, p_1 and p_2, and χ^2 values derived from the data contained in these reports. Since these studies have used different methods of quantifying the Hb fractions, the range of the phenotypic classes varies slightly by study. The results show an excellent fit between these observations and the predictions of the model; none differs significantly from the expected distribution. Thus, the proportion of HbS in heterozygotes in these populations may be considered to be binomially distributed.

What do G_1 and G_2 represent? One hypothesis would suggest that they are β-thalassaemia-like genes which selectively diminish β^S but not β^A production; a binomial distribution of the proportions of HbS would result. No β-thalassaemia gene affecting the synthesis of a β-chain structural mutant has, however, been definitely demonstrated. Moreover, higher HbA_2 levels might be expected but only modest elevations have been observed in heterozygotes with low proportions of HbS. There seems to be no evidence to support this possibility. A second hypothesis proposes that variations in the number of β-globin gene loci account for variations in the proportion of HbS in hererozygotes[15]. Both the available genetic data[16–18] and direct measurement of the number of β-globin genes[19] indicate, however, the existence of only a single β-chain locus.

The simplest remaining explanation is that G_1 and G_2 represent the number of active α-globin structural genes. Under this hypothesis, G_1 and G_2 would be chromosomes with

Table 2 Observed distributions of the proportions of HbS in sickle-cell trait and Hardy–Weinberg expectations: various populations

Reference		Population	Number			Genotypes		'Gene' frequencies		χ^2
					G_1G_1	G_1G_2	G_2G_2	p_1	p_2	
6		US negroes			HbS < 30%	HbS 30–38%	HbS > 38%			
			70	Obs.	5	29	36	0.279	0.721	0.066 $P>0.70$
				Exp.	5.4	28.1	36.4			
7		US negroes			HbS < 30%	HbS 30–35%	HbS > 35%			
			69	Obs.	3	15	51	0.152	0.848	1.712 $P>0.10$
				Exp.	1.6	17.8	49.6			
8		Brazilian negroes			HbS < 30%	HbS 30–38%	HbS > 38%			
			60	Obs.	3	13	44	0.158	0.842	2.100 $P>0.10$
				Exp.	1.5	16.0	42.5			
9		Brazilian negroes			HbS < 30%	HbS 30–38%	HbS > 38%			
			272	Obs.	2	38	232	0.077	0.923	0.104 $P>0.70$
				Exp.	1.6	38.8	231.6			
10		Brazilian negroes			HbS < 30%	HbS 30–36%	HbS > 36%			
			153	Obs.	0	31	122	0.101	0.899	1.944 $P>0.10$
				Exp.	1.6	27.9	123.6			
11		Nigerian negroes			HbS < 28%	HbS 28–36%	HbS > 36%			
			244	Obs.	52	135	57	0.490	0.510	2.795 $P>0.05$
				Exp.	58.5	121.9	63.5			
12	a	Nigerian negroes			HbA > 70%	HbA 65–70%	HbA < 65%			
			79	Obs.	39	34	6	0.709	0.291	0.144 $P>0.70$
				Exp.	39.7	32.6	6.7			
	b	Ghanian negroes	63	Obs.	17	33	13	0.532	0.468	0.169 $P>0.60$
				Exp.	17.8	31.3	13.8			
13		Saudi Arabian caucasians			HbS < 30%	HbS 30–38%	HbS > 38%			
			109	Obs.	35	56	18	0.578	0.422	0.308 $P>0.50$
				Exp.	36.4	53.2	19.4			
14		Indians (Irula)			HbS < 30%	HbS 30–35%	HbS > 35%			
			79	Obs.	70	9	0	0.943	0.057	0.288 $P>0.50$
				Exp.	70.3	8.5	0			
*	a	Indians (Mulla Kurumba)			HbS < 30%	HbS 30–34%	HbS > 34%			
			55	Obs.	7	30	18	0.400	0.600	1.023 $P>0.30$
				Exp.	8.8	26.4	19.8			
	b	Indians (Paniyan)	39	Obs.	27	10	2	0.821	0.179	0.654 $P>0.40$
				Exp.	26.3	11.5	1.3			
	c	Indians (Sholiga)	22	Obs.	21	1	0	0.977	0.023	0.012 $P>0.90$
				Exp.	21.0	1.0	0			

*G. Brittenham *et al.*, unpublished

1 and 2 active α-globin loci, respectively. G_1G_1 would correspond to an α^A/α^A genotype, G_1G_2 to $\alpha^A/\alpha^A\alpha^A$, and G_2G_2 to $\alpha^A\alpha^A/\alpha^A\alpha^A$. A net α-chain production deficit would be found in individuals with less than 4 α genes. In at least some cases, α-thalassaemia (α-chain production deficit with unbalanced α/β synthesis) results from deletion of the α-chain gene locus. It has been demonstrated that a low proportion of HbS is found in some heterozygotes with α-chain deficiency due to α-thalassaemia[20,21]. Calculation of the absolute amounts of the Hb fractions per cell (MC[HbA] and MC[HbS]) demonstrates that the absolute amount of HbA does not differ significantly between those with high or low percentages of HbS. Only the amount of HbS (MC[HbS]) is decreased in those with a low percentage of HbS[22]. This result is evidence that α chains have a greater affinity for β^A chains than for β^S chains, a hypothesis that was suggested over a decade ago[23] and is supported by recent *in vitro* data[24]. Thus, the proportion of HbS can reflect the availability of α chains, which can be, in turn, a function of active α-globin gene number.

α-Thalassaemia may not be an invariant finding in those individuals with a low proportion of HbS. A recent study has presented evidence that α/β synthesis may be balanced in individuals with two or three α-globin genes[25]. Furthermore, balanced globin synthesis has been reported in two heterozygotes with 25 and 28% HbS[11]. In the absence of other conditions affecting globin synthesis, a low proportion of HbS can indicate a deficit in α-globin chains due to a decreased number of active α-globin structural genes, with or without an associated imbalance in globin synthesis (α-thalassaemia).

If the proposed interpretation of the genetic model is correct, there should be a close correspondence between model predictions of α^A and $\alpha^A\alpha^A$ gene frequencies and estimates by other techniques. The level of Bart's Hb (γ_4) in cord blood specimens has been used to estimate the number of α-chain loci. In the same US black population reported in study 2 (ref. 7; Table 2), analysis of 14,058 cord blood samples has provided frequencies of 0.162 for α^A and 0.838 for $\alpha^A\alpha^A$ genes[26]. These values are almost identical to the estimates of 0.152 for α^A and 0.848 for $\alpha^A\alpha^A$ derived from analysis of the observed distributions of the percentages of HbS (Table 2). Other evidence, including measurement of the proportion of α-chain variants[18], the number of α-chain variants in certain individuals[18], and direct measurement of the number of α-globin genes in DNA hybridisation experiments[19], indicate that the α-chain locus is duplicated in some populations. Finally, recent family studies have provided further evidence that the proportions of β-chain variants in heterozygotes are a function of the number of α-chain loci[27].

The existence of a chromosome with no active α-chain loci has recently been demonstrated in American blacks[26,28] and is well documented in Asians[29]. The genetic model may be extended by letting G_0, at frequency p_0, represent this chromosomal condition. For a population in Hardy–Weinberg equilibrium and having three types of chromosome, with no, one and two α-chain loci, the genotype frequencies are given by the binomial expansion $(p_0+p_1+p_2)^2$ as shown in Table 3. The distributions of the proportions of HbS reported for the heterozygotes of 11 of 12 Guinean and Angolan populations[30] fit the predictions of the three chromosome type model (data not shown, available from the author). This result must be interpreted with caution, however, because the conditions of this study[30] precluded detection of hydrops fetalis, HbH disease or α-chain variants.

Table 3 Phenotypes and genotypes: model with three genetic units (G_0, G_1 and G_2)

Phenotype (% HbS) Mean	Range	Model genotype	Frequencies
(Hydrops fetalis)		G_0G_0	p_0^2
17	(15–19)	G_0G_1	$2p_0p_1$
(HbH disease)			
25	(20–29)	G_0G_2, G_1G_1	$2p_0p_2+p_1^2$
33	(30–36)	G_1G_2	$2p_1p_2$
40	(37–50)	G_2G_2	p_2^2

The genetic model presented here suggests that human populations are polymorphic for the number of active α-globin genes. With differing proportions of HbS in sickle-cell trait, the absolute amount per cell of HbS, but not HbA, varies, supporting the *in vitro* evidence that α chains have a greater affinity for β^A chains than for β^S chains. The proportion of HbS can, therefore, reflect the availability of α chains which, in turn, can be a function of the number of α-globin genes. By using the proportion of HbS as an indicator of α-globin number and postulating that populations are in Hardy–Weinberg equilibrium for chromosomes with two, one and no α-chain loci, the distribution of the proportion of HbS can be accounted for in 24 of the 25 populations reviewed.

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Absolute visual threshold and scotopic spectral sensitivity in the tawny owl *Strix aluco*

IT has long been assumed from considerations of the behaviour of owls that the vision of these species is much more sensitive than that of man. Experimental determinations of the absolute visual sensitivity of these birds have provided evidence in support of this view, suggesting that visual sensitivity in owls is of the order of 10 to 100 times (+1.0 $\log_{10}$ to +2.0 $\log_{10}$ units) that of man[1,2]. Owing to calibration problems, however, these values were probably inaccurate by at least a factor of 10 (refs 1, 2). I report here that in at least one highly nocturnal owl species, visual sensitivity is only slightly higher than that of man, and that the increased sensitivity can be attributed to optical factors rather than to any increase in sensitivity at the retinal level. This suggests that retinal mechanisms in both man and owl have reached the ultimate in sensitivity for the duplex retinas of terrestrial vertebrates.

Determinations of human absolute sensitivity in ideal experimental conditions suggest that once a photon has been absorbed by the photoreceptors, the human retina comes close to the theoretical limit of sensitivity[3,4]. Hecht, Shlaer and Pirenne[3] have shown that single photoreceptors can respond to the absorption of a single quantum of light and that the retina as a whole is capable of detecting small brief stimuli when only about 10 light quanta are absorbed by the receptors. The emphasis in these figures is, however, on absorbed quanta and a sensitivity increase over that of man could be achieved at the retinal level by increasing the probability of quantum catch in the receptors. This could be effected by enlarging the photoreceptor outersegment and/or by increasing the density of pigment contained therein[5].

Considerable improvement in sensitivity of the eye as a whole, as opposed to that of the retina alone, could be achieved by an increase in the relative proportion of quanta falling upon per unit retinal area for the given stimulus external to the eye. (Approximately 90% of quanta incident on the human cornea are absorbed or scattered before reaching the retinal receptors[4,6].) This could be achieved by increasing the light gathering power (photographic speed) of the eye and by increased transmission of light through the optic media.

I determined the absolute sensitivity threshold to achromatic light and the scotopic spectral sensitivity of two tawny owls, *Strix aluco,* using a training procedure designed to produce a high degree of behavioural control by the visual stimuli close to threshold. For comparison, the absolute thresholds of two human subjects were determined in as near identical conditions as possible.

The owls were maintained as described previously[7], and a two-choice simultaneous discrimination procedure with appetitive reinforcement was used. The training procedure and apparatus were modified versions of those used previously with the same subjects[7,8]. One important modification was the addition of a gentle air flow directed towards the bird around the response bars while a trial was in progress, and around the food dish during reinforcer presentation. This air flow was used as a cue to increase behavioural control of the animals while responding in total darkness[9]. All surfaces inside the response chamber were painted matt black. The whole apparatus (which was mounted in a light-proof room) was made light tight and during training was covered by a large bag of heavy gauge black polythene sheeting.

Table 1 shows absolute threshold data for achromatic stimuli for the owls and humans after different length dark-adaptation periods. For the owls training and testing lasted approximately 12 months. Sessions in which no light was presented produced a chance level of discriminative performance suggesting that the animals' behaviour was not under the control of stimuli other than the stimulus lights.

As found in other vertebrates dark adaptation in the owl is close to asymptote after 30 min. Comparison with previously published[4] human absolute threshold data for extended light sources (mean of 22 subjects = 0.75×10^{-6} cd m^{-2} range 0.4–2.0×10^{-6} cd m^{-2}) shows that the human thresholds recorded here were within the normal range. Clearly, the behaviourally determined absolute sensitivity of the owl is not as superior to that of man as has commonly been supposed. The normal range within which human absolute thresholds lie covers approximately 1.0 $\log_{10}$ unit, and the data presented here suggest that while the absolute threshold of the owl is probably below that of man the two species show considerable overlap in the distribution of their thresholds. The broadness, compared with that of man, of the owl scotopic spectral sensitivity function (Fig. 1), which has also been found electroretinographically[11], is probably attributable to the slightly increased *in situ* rod visual pigment density in the owl. The spectral locus of maximum sensitivity corresponds with the locus of maximum absorption of owl rod visual pigment (λ_{max} 503 nm). (Bowmaker, J.K. and Martin, G.R. in preparation).

It does not seem that the increase in owl sensitivity compared with that of man, is attributable in any large measure to differences in either pigment density or retinal neural mechanisms in the two species. Consideration of the light-gathering power of the tawny owl's tubular shaped eye suggests that as in the cat[12] (where the position is complicated by the presence of a tapetum) the difference in the light-gathering power of the human and owl eye is sufficient to account for the observed difference between owl and human absolute sensitivity. The minimum *f*-number (maximum pupil diameter) of the owl eye is 1.30 (in preparation), while that of man[13,14] is 2.10 producing a retinal illumination in the owl approximately 2.6 times that of man (compare mean absolute sensitivity difference (two owls compared with two humans) of approximately 2.5-fold). Thus it can be concluded that the human extrafoveal retina is of approximately equal sensitivity to that of other vertebrates considered to be highly nocturnal. The data also suggest that the human retina, along with those of the cat and the owl, has indeed attained the absolute limit of

Table 1 Absolute visual sensitivity threshold data for two humans and two tawny owls following various dark-adaptation periods

	Dark-adaptation period (min)	Threshold $\log_{10}$	Mean $\log_{10}$	Mean threshold calibrated (cd m^{-2})	Threshold relative to human
Owl (01)	30	3.54	3.56	0.84×10^{-6}	$\times 1.45(+.16 \log_{10})$
	60	3.64			
	90	3.49			
Owl (02)	30	3.96	4.04	0.28×10^{-6}	$\times 4.36(+.64 \log_{10})$
	60	4.17			
	90	4.00			
Human *a*	30	3.50	3.40	1.22×10^{-6}	
Human *b*	30	3.38			

Thresholds are shown in the arbitrary $\log_{10}$ units from which the calibrated values were determined. Subjects were dark adapted in the apparatus. The only light present throughout training and testing was the stimulus light. Length of stimulus presentation was determined by the latency of the subjects' response and was rarely more than 5 s even during blank probe trials when no stimulus light was presented. Reinforcers were presented during 10-s intertrial intervals signalled by a buzzer. Forty trials per daily session were used with a left–right Gellerman sequence and stimulus intensities presented in a quasi-random order according to a method of constant stimuli. Psychophysical functions (% trials correct per stimulus intensity) were constructed for each subject using between 40 and 100 trials per intensity and these were interpolated at the 75% correct point to give threshold values. Threshold data were accumulated after extensive training. The human subjects (male aged 27 yr; female, 26 yr) viewed the stimuli without fixation through a hole cut in the back of the owls' response chamber (stimulus subtense, 23 × 15°) and responded by closing a microswitch. Extensive practice was given. Fifty trials were recorded at each stimulus intensity and thresholds determined as for the owls. The stimulus light (correlated colour temperature 2850 K) was provided by a quartz halogen lamp (GEC 450, under-run from a constant current supply) back projected onto 12 × 8 cm (angular subtense at bird's perch, 48 × 34°) ground glass panels by an optical system similar to that described by Muntz and Northmore[10]. Light entered the apparatus through a 5-mm diameter hole (placed at the focal point of one of the lenses) in a light-proof box mounted behind the stimulus panels.

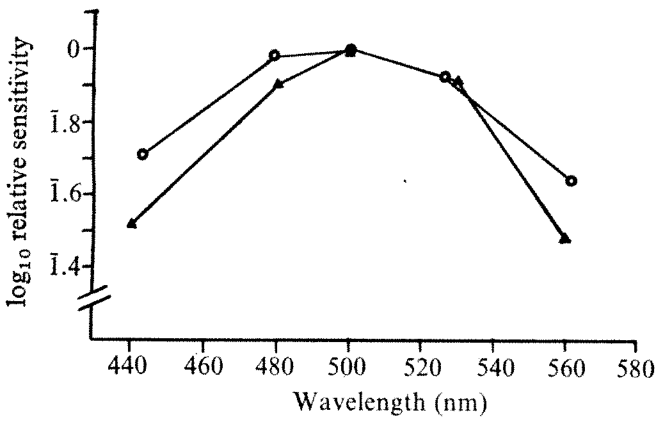

Fig. 1 Behaviourally determined scotopic spectral sensitivity of the tawny owl (○) (mean of two birds). Thresholds were determined using interference filters and the same procedure and apparatus as in the determination of absolute sensitivity to achromatic stimuli. For comparison the human CIE V_λ' function is also shown (▲). Sensitivity is expressed according to an equal quantum intensity base. For calibration of stimuli the following were used: an Hilgar–Shwartz F.T. 17 thermopile placed at the first focus of the optical system to determine the relative energy available through the interference filters (Balzars, B40) and the density of high value neutral density filters *in situ*; an EMI photomultiplier with entrance window placed against the stimulus panels was used to determine the *in situ* density of the low value neutral density filters; a Shimadzu scanning spectrophotometer to determine the spectral transmission of the interference filters. An SEI photometer was used to determine the absolute luminance values of the stimulus panels with filters removed from the beam, and stimulus values at threshold were determined by calculation from the calibrated filter values.

sensitivity, at least for those terrestrial vertebrates with duplex retinas in which some kind of pattern vision is required at low light levels.

Man is typically used as the yardstick for interspecies comparisons of sensory capacities. To gain some appreciation of the adaptive significance of such capacities for the owl, however, a comparison with man is not particularly appropriate. Of more value is the comparison of the owls' sensitivity with that of a diurnal bird, such as the pigeon, *Columba livia*. This species, which is known to roost immediately on being placed in darkness, has an absolute sensitivity approximately 2.0 $\log_{10}$ unit (that is, 100-fold) lower than the owl[15]. This difference in sensitivity is probably attributable to a difference in retinal mechanisms since retinal illumination in the pigeon is approximately equal to that of man[16] (from observation of pupil diameter). Thus, although the vision of owls does not seem to be as highly sensitive as previously supposed, the sensitivity of these birds can be seen nevertheless to provide them with considerable behavioural advantage over other (diurnal) avian species at low light levels.

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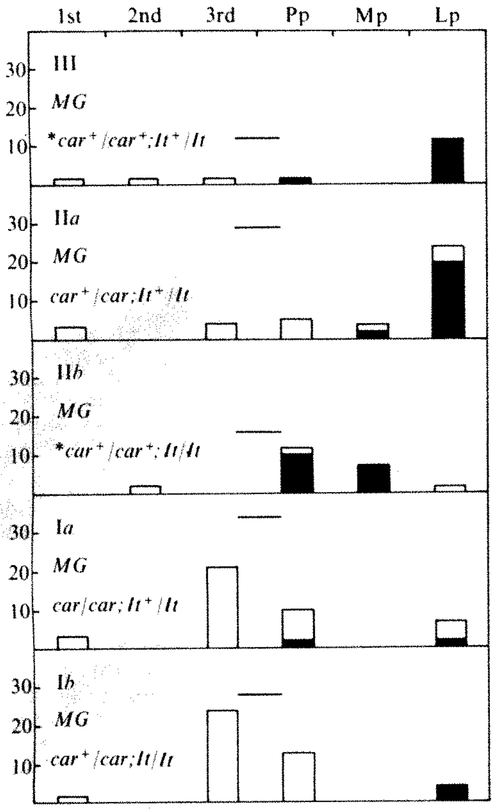
Maternal effects determine effective lethal phase of *carnation–light* synthetic lethal in *Drosophila melanogaster*

GENETIC conditions which preclude development to the reproductive stage (lethal factors) include dominant and recessive Mendelian units, genomic imbalance and gene interaction. Determination of the ontogenetic phase at which each lethal factor inflicts atypical development and death (effective lethal phase[1]) provides an opportunity for study of specific genetic requirements for normal development. I describe here maternal effects which determine the effective lethal phase (ELP) of a synthetic lethal[2] produced by the interaction of two non-lethal eye-colour mutations, *carnation* (*car*) and *light* (*lt*), in *Drosophila melanogaster*.

Penetrance of the lethal interaction of the *car* (position 62.5 on the X chromosome) and *lt* (position 55.0 on the second chromosome) genes is complete such that all *car-lt* double mutants (*car*/*car* : *lt*/*lt* and *car*/Y : *lt*/*lt*) die before eclosion. Both mutations cause a general reduction in pteridines and ommochrome precursors in larvae and adults when compared with wild type. Compound accumulations are similar to wild type during pupal stages[3].

The influence of the maternal genotype on the ELP was determined by examining the stage of developmental arrest of *car-lt* offspring from matings in which the number of car^+ and/or lt^+ genes varied in the mother. As Fig. 1 shows, a general trend is apparent—as the number of maternal car^+ and lt^+ genes increases, the later developmental arrest occurs in *car-lt* offspring. Yet, a comparison of data presented for crosses II*a* and II*b* indicates that the stage of developmental arrest is not solely dependent on the number of maternal car^+ and/or lt^+ genes. Females homozygous for *lt* produce synthetic lethal offspring which die at early pupal stages (cross II*b*), whereas those from females heterozygous at *car* and *lt* loci die at late pupal stages. A synergic relationship therefore exists between maternally derived car^+ and lt^+ gene products. The difference in ELP in offspring from crosses I*b* and II*b* suggests that the car^+ gene is semidominant with respect to lethal phase determination. The appearance of the semidominant behaviour of car^+ in crosses II*a* and III may be masked by the fact that the late pupal stage is the final stage obtainable by *car-lt* double mutants regardless of the maternal genotype. A test cannot be made of the dominance relations of lt^+ with respect to survival of synthetic lethal offspring. A comparison of crosses I*a* and I*b* indicates that maternal car^+ and lt^+ gene products have a similar influence on determination of the lethal phase of larvae. The relative infrequency of second-instar lethality is in agreement with other investigations[4,5]. No differences between sex and lethal phases were observed in any cross. Whether activity of maternal car^+ or lt^+ genes eliminates the cause of larval death of *car-lt* double mutants or merely prolongs the onset of a common developmental crisis is not known. Synthetic lethal larvae from crosses I*a* and I*b* show no gross abnormalities. Pharate adults from crosses II*a* and III, however, show abnormal development of internal genital disk derivatives (males) and incompletely developed abdominal tergites (males and females).

It is generally agreed that each lethal factor defines a particular ELP (monophasic) as embryonic, egg-larval



*attached-X chromosome

Fig. 1 Number of *car-lt* double mutants (shaded) and unclassifiable (open) offspring dying at various stages from crosses in which the maternal genotype (MG) included various numbers and/or combinations of car^+ and/or lt^+ genes. The paternal genotype was *car*/Y; lt^+/lt (plus appropriate marker genes described below) for each cross. Eggs collected from females (aged 2–6 d) were placed in culture tubes (6×50 mm), two per tube, containing 0.1 ml of standard medium. Each tube was examined periodically and scored as to condition of eggs (hatched or unhatched), larvae (instar: 1st, 2nd or 3rd) and pupae (stage). Pupae which failed to eclose within 15 d of egg collection were scored as to the stage of developmental arrest: prepupa (Pp), midpupa (Mp, pupation to onset of bristle pigmentation), late pupa (Lp, bristle pigmentation to pupa ready to emerge). Dead larvae, Pp, or pupae were classified as to genotype when possible by appropriate marker genes (*y, lt, B, f, Cy*) as described by Lindsley and Grell[12]. Fertility ratios were determined thus allowing computation of the expected number of *car-lt* double mutants for each cross. This number is represented by a horizontal line (————) in each component of the figure.

(boundary), larval-pupal (boundary) or late pupal[4] in which the degree of genetic change is inversely related to the duration of survival[6]. A direct relationship between genetic complement and time of death was demonstrated by Shannon *et al.*[7] where a consistent ELP is observed within a given genetic complementation group. A multiphasic lethal pattern is sometimes observed when environmental factors (crowding and so on), genetic background, variations in pentrance and maternal genotype cause some individuals to die at a stage earlier than the characteristic lethal phase[4,6–8]. Li[9] reported maternal effects determining survival of all offspring of *Plexate, Minute-1, plexus* and haplo-IV females. Each case, however, seems to be related directly to the mother's ability to produce functional eggs of all Mendelian classes. Because fertility ratios computed in this study show no consistent relationship to the number of maternal car^+ or lt^+ genes, it can be assumed that the ability of the females used to produce functional eggs *per se* is unrelated to the time of developmental arrest in *car-lt* double mutants. Rather, when sufficient contributions are made by maternal car^+ and lt^+ genes, *car-lt* double mutants transcend the larval lethal phase (crosses II*a*, II*b* and III) and enter the radically different developmental phase of metamorphosis.

Maternally derived lt^+ gene products which alter Malpighian tube pigmentation in *lt/lt* offspring from lt^+/lt mothers[10] may be involved in lengthening survival time in *car-lt* double mutants. It is improbable, however, that these products directly lengthen survival beyond pupation (as in crosses II*a* and III), for the maternal influence on pigmentation ceases before pupation[11]. The chromatographic-fluorometric procedure used to assess the pigment maternal effect resulting from activity of the lt^+ gene was used to determine whether a similar maternal effect is associated with car^+. Results were inconsistent with such an effect.

The viability of *car/car* or *lt/lt* genotypes indicates that their alleles (car^+ or lt^+) are not necessary for normal development unless homozygosity for the mutant allele exists at the other locus. In these cases survival depends on the presence of car^+ or lt^+ gene products by at least the early third-instar stage. In *car-lt* double mutants these products are maternally supplied whereas in survivors (car^+/car : *lt/lt* or *car/car* : lt^+/lt for example) these gene products are supplied by the organism's own genome as well as that of the mother.

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Age- and region-dependent concanavalin A reactivity of chick wing-bud mesoderm cells

MORPHOGENETIC potential of embryonic chick limb mesoderm cells declines with time beginning at approximately stage 24 of development[1]. This decline occurs in a proximo-distal direction[2]. Accompanying the loss of morphogenetic potential there is an age-dependent decline in the ability of limb and axial mesoderm to sort from one another in a randomised mixture of cells[1,3,4]. Due to the age-dependent decline in both morphogenetic potential and the sorting ability of the limb mesoderm cells, it has been suggested that the decline in morphogenetic potential may be related to changes occurring at the cell surface[5]. Cell surface carbohydrate moieties have been postulated to play an integral part in cellular interactions[6]. Because of their high degree of carbohydrate-binding specificity[7], plant lectins, such as concanavalin A (con A), *Ricinus communis* agglutinin (RCA), and wheat germ agglutinin (WGA), have been used to monitor cell surface changes which occur during development[7–13]. These changes manifest themselves as differences in lectin-induced agglutinability of cells, and changes in number and distribution of lectin binding sites on the cell surface. This study was designed to determine if there were cell surface changes accompanying the decline in morphogenetic potential of the limb mesoderm cells, which could be detected by the use of plant lectin probes. We report here that there is a slight increase in RCA-induced agglutination of chick wing-bud mesoderm cells with increasing developmental age. There is also a significant age-dependent, proximo-distal decline in the susceptibility of chick wing-bud mesoderm cells to con A-induced agglutination between stages 19 and 26 of development. As such, there is a spatial as well as a temporal correlation between the decline of con A agglutinability and the decline of the morphogenetic potential of the chick limb-bud mesoderm cells.

Zwilling has suggested that the events of limb development can be divided into two phases: a morphogenetic phase and a phase of cytodifferentiation[1]. Limb mesoderm cells have a set of properties during the morphogenetic phase which are responsible for the establishment of general limb form[1]. The ability of limb mesoderm to express these morphogenetic properties or potential declines in a proximo-distal direction from a peak at stage 19–20, until the morphogenetic potential is lost at about stage 26 (refs 1, 2). During the interim period, the morphogenetic potential of the limb mesoderm cells in a single limb bud is region-dependent[2]. Table 1 shows that the agglutination of chick wing-bud mesoderm cells with WGA (125 μg ml^{-1}) is nearly complete and not age-dependent. RCA (125 μg ml^{-1}) induces slightly greater agglutination of cells from older wing buds than of those from younger wing buds. In contrast, treatment with con A at the same concentration induces a significantly greater degree of agglutination of mesoderm cells derived from whole stage 19–20 wing buds than it does of mesoderm cells derived from whole stage 25–26 wing buds. The disparity between the morphogenetic potential of proximal and distal mesoderm in a single limb bud is greatest at stage 25 of development[2] (Table 1). Limb mesoderm cells from the proximal third of a stage 25 wing bud are significantly less susceptible to con-A induced agglutination than are those from the distal third. Furthermore, the difference in susceptibility to con A-induced agglutination between mesoderm cells from the proximal and distal thirds of the stage 25 wing-bud, is greater than that between cells derived from whole stage 19–20 and 25–26 wing buds. This is not surprising since whole stage 25 and 26 limb buds contain mesodermal cells of relatively high con A agglutinability in their distal region.

Table 2 Specific binding of ^{3}H-con A to stage 25 wing-bud mesoderm cells

Region of wing bud furnishing mesoderm cells	Specific binding of ^{3}H-con A (c.p.m. per mg protein)
Distal third	20,616±910 (n = 5)
Proximal third	20,844±700 (n = 7)

Single cell suspensions of proximal and distal stage 25 wing mesoderm were obtained as described in the legend for Table 1. Binding experiments were performed at 0 °C to minimise endocytosis of bound con A. The binding reaction was done in a 1 ml volume containing 5 × 10^{6} cells, 125 μg unlabelled con A and 1 μCi ^{3}H-con A (specific activity 41 Ci mmol^{-1}) (New England Nuclear). Controls contained the same components plus 0.1 M α-methyl-D-glucose (Sigma) to indicate the non-specific binding of con A. The amount of binding observed in the controls was subtracted from the values of total binding to give the amount of specific binding. The tubes containing the reaction components were kept at 0 °C for 1 h with mixing at 15-min intervals, after which they were centrifuged at 2,500 r.p.m. for 5 min. After the supernates were removed, the pellets were washed three times in 2 ml of ice cold Tyrode's solution to remove any unbound ^{3}H-con A. The pellets were then dried and dissolved in 1 ml of 1 M NaOH. Aliquots were taken for scintillation counting and protein determinations by the Lowry method[17]. Values are mean ± s.e.m.

It is not yet possible to draw conclusions regarding a causal relationship between the cell-surface changes (suggested by the decline in con A-induced agglutinability) and the loss of the morphogenetic potential of the limb mesoderm cells. There is evidence, however, of a positive temporal as well as spatial correlation between these two phenomena.

To determine the basis of the differential con A reactivity between the cells from the proximal third and those from the distal third of the stage 25 chick wing bud, the relative con A binding capabilities of the cells from these two regions were assayed using ^{3}H-con A. These experiments indicate that there is no significant difference between the amounts of ^{3}H-con A bound by the cells from these two regions (Table 2). This is in contrast to a decline with increasing developmental age, in the amount of con A bound by normal mouse limb mesoderm cells between days

Table 1 Correlation of lectin-induced agglutinability of whole stage 19–20 and stage 25–26 wing-bud mesoderm cells and mesoderm cells from proximal and distal thirds of stage 25 wing buds with their morphogenetic potential

Region and stage[18] of wing bud furnishing mesoderm cells	%Specific agglutination with RCA*	%Specific agglutination with WGA*	%Specific agglutination with con A*	Morphogenetic potential†
Whole 19–20	25.2±0.7‡	98.2±0.2§	64.4±1.7‖	96.5
Whole 25-26	33.1±2.0‡	98.7±0.3§	51.8±2.2‖	37.8
Distal third 25	—	—	68.2±2.1¶	100.0
Proximal third 25	—	—	44.5±3.2¶	0.0

*Mesoderm from whole wing buds of stage 19–20 and stage 25–26 white leghorn chicken embryos and mesoderm from the proximal or distal third of stage 25 wing buds was collected by dissecting the wing buds from the embryos in a dish containing Tyrode's solution. The proximal and distal thirds of the stage 25 wing buds were kept separate. The whole wing buds or the proximal or distal segments were transferred to depression dishes in which they were incubated with 2% trypsin (Nutritional Biochem.)+1% pancreatin (Nutritional Biochem.) in Tyrode's for 15–20 min at 37 °C to loosen the ectoderm. The ectoderms were removed, and after washing with Ca^{2+}-free Mg^{2+}-free Tyrode's (CMF), the mesoderm was cut into fragments and incubated in CMF containing 0.02% EGTA (ref. 9) (Sigma) at 37 °C for 45 min. After this treatment, the EGTA solution was replaced with Tyrode's containing 10 μg ml^{-1} DNase (ref. 9) (DNase–Tyrode's) (Nutritional Biochem.). After transferring the mesoderm fragments to Falcon plastic test tubes containing 2 ml of DNase–Tyrode's, single-cell suspensions were obtained by mild agitation. The number of cells in each suspension was determined with a haemocytometer and adjusted so that after addition of the appropriate plant lectin (Sigma) and its specific sugar inhibitor (Sigma) in DNase–Tyrode's to control cultures, and the appropriate plant lectin in DNase–Tyrode's to the experimental cultures, each gave a final cell density of approximately 2.5 × 10^{6} cells ml^{-1} in a total volume of 3 ml. The final concentration of plant lectin was 125 μg ml^{-1}. To control for nonspecific agglutination, α-methyl-D-glucose, *N*-acetyl-D-glucosamine, or α-lactose (all Sigma) were added to control cultures containing con A, WGA, and RCA respectively. The final concentration in the control cultures of these specific inhibitors was 0.1 M. The cells were incubated in siliconised 25 ml Erlenmeyer flasks for 30 min at 37 °C in a gyratory shaker water bath, operating at 70 r.p.m. After incubation, samples were examined in a haemocytometer and single cells remaining counted. The disappearance of single cells from the cell suspensions was used as a measure of cell agglutination.

*Percentages of nonspecific agglutination were subtracted from the values of total agglutination to give the percentages of specific agglutination. Values are mean±s.e.m.

†Percentage of grafts, containing mesoderm cells taken from specific stage and region, which formed complex limb structures as determined by the assay of Zwilling[16]. Values for whole limbs obtained percentages for component parts[2].

‡Whole stage 19–20 significantly different from whole stage 25–26. (Student's t test, $P < 0.05$).

§Not significantly different (Student's t test).

‖Whole stage 19–20 significantly different from whole stage 25–26 (Student's t test, $P < 0.001$).

¶Distal third of stage 25 significantly different from proximal third of stage 25 (Student's t test, $P < 0.001$).

11 and 12 of development[14], and an increase in the number of binding sites for con A with increasing developmental age in embryonic chick neural retina cells between days 8 and 10 of development[11]. In the chick limb, mouse limb, and chick retina systems, these changes in the number of con A binding sites with increasing developmental age are all accompanied by a decrease in con A-induced agglutinability. Therefore, it seems that some cell membrane or surface property, other than the number of con A binding sites, is responsible for the observed differences in con A-induced agglutinability. The mechanism responsible for the differing response to con A treatment in our system may involve the lateral mobility of the con A binding sites within the plane of the plasma membrane since the clustering of these sites has been shown to accompany increased agglutination in other systems[11,15].

Morphogenesis involves the interaction of large numbers of cells. Since a primary site of such interactions is at the cell surface, an understanding of the cell-surface changes which occur during development as well as how they relate to changes in the morphogenetic potential of cells, will be of significance to our understanding of morphogenesis in general.

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Monogamous T helper cell

HUMORAL antibodies are produced by lymphocytes of bone-marrow or bursal origin (B cells). Some antigens are apparently capable of stimulating B cells and inducing a response directly but many other antigens are dependent on the cooperative help of lymphocytes of thymic origin (T cells). There are at least two forms of such cooperation between B and T cells. One type of cooperation is 'non-specific' where T cells once activated by specific antigen release a nonspecific factor which can then facilitate the responses of B cells to certain other non-related antigens[1]. The other well studied cooperative mechanism is known as 'specific cooperation' and classically this has been analysed with the use of 'hapten-carrier' systems[2]. The exact nature of this cooperative event is not known. It may be that co-operation occurs through widely diffusible specific factors released by T cells, or alternatively the cooperating T and B cells may need to come into close or direct contact. We have studied helper T-cell function in a microculture system with the aim of determining whether a single helper T cell could activate one or many B cells. Experiments of this type have previously been performed by repopulating mice with limiting numbers of immunocompetent cells and examining the resulting foci of antibody activity. When T cells were the limiting population some have found these foci to be heterogeneous[3] and others have found them to be monoclonal[4,5]. Similar *in vitro* studies have also suggested marked restriction in the B-cell response where T helper cells were limiting[6]. Here we present direct evidence to support the findings demonstrating restriction. We conclude that in the conditions used, one specific helper T cell cooperated with one B cell only.

Table 1 Number of clones observed in each well compared with numbers expected for random distribution of T cells

No. of clones	0	1	2	3	4	5
No. of wells observed to contain the following numbers of TNP-specific B-cell clones*	16	26	12	4	1	0
No. of wells estimated to contain the following numbers of TNP-specific B-cell clones if 1 T cell activates 1 B cell†	19.28	21.56	12.06	4.53	1.28	0.28

*Analysis of the number of wells containing different numbers of B cell clones from the 59 samples analysed in Fig. 2.

†If we hypothesise that one T cell activates one B cell then the clones would be distributed according to the Poisson distribution of T cells getting into wells. A total of 66 clones as observed would be expected to distribute as shown.

The culture system used in the experiments was the

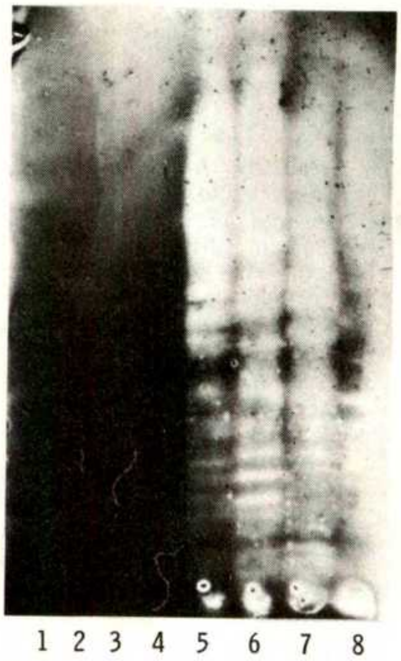

Fig. 1 Anti-TNP spectrotypes obtained from supernatants of *in vitro* microcultures. Antibody was visualised by overlaying the gel with haptenated red blood cells and complement[15]. Microcultures were performed as before[5]. B cells were obtained from mice primed three months previously with 500 µg of TNP-ovalbumin in Freund's complete adjuvant and subsequently boosted twice with 200 µg of soluble antigen. The last boost was performed two weeks before the experiment. The spleen cells from these mice were treated with anti θ and complement to remove T cells. T cells were derived from mice primed one month previously with 250 µg of KLH in Freund's complete adjuvant and boosted with 200 µg of soluble KLH 6 d before killing. The spleen cells from these mice were enriched for T cells by passage through nylon wool. 1.5×10^5 B cells were cultured with antigen (TNP-KLH) in multiple wells containing 10 µl of medium. Samples 1–4 were from four cultures having no T cells. Samples 5–8 were from wells having 5×10^4 T cells per well.

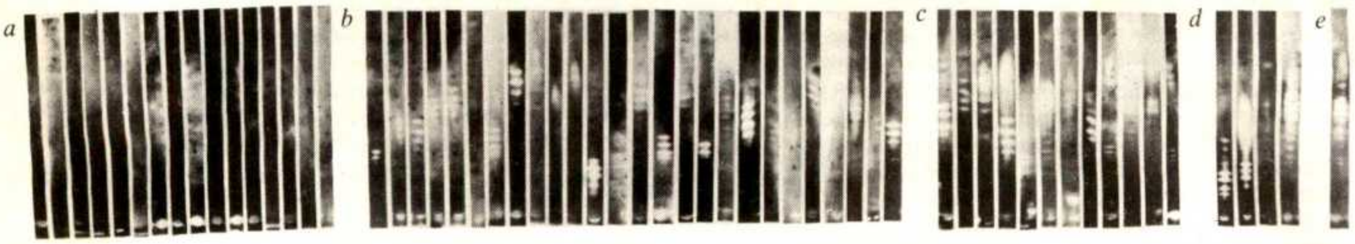

Fig. 2 Anti-TNP spectrotypes from supernatants of *in vitro* microcultures. Antibody visualisation and culture conditions are described in Fig. 1 but in this case 1.5×10^5 B cells and 2.5×10^3 T cells per well were used. 59 individual supernatants were analysed and samples grouped according to the numbers of different spectrotypes (a,b,c,d,e, = 0,1,2,3,4 spectrotypes respectively).

microculture system devised by Lefkovits[7]. The technical procedures adopted for studying the functions of single helper T cells have been described before and it was shown that one single helper T cell was sufficient to activate a B cell response to antigen[8]. Levels of hapten specific B cells were used such that each well contained more than 5 B cells and then T cells were titrated to a level such that many wells would contain 0 or 1 specific helper T cell. After 7 d of culture the supernatants from the cultures were analysed by isoelectric focusing (IEF). By this method different spectrotypes are interpreted as representing the products of different B-cell clones. Figure 1 shows that when no T cells were added all wells were negative, and that when a high number of T cells were added to each well the responses were so heterogeneous that the exact number of clones could not be accurately counted but we can say that there were more than five per well. At a T-cell input of 2.5×10^3 T cells per well, then 16 out of 59 wells which we sampled were negative. With this number of negatives it can be estimated that the majority of the positive wells would contain only one helper T cell. The number of clonal products observed in each of the positive wells of this tray were then counted. In Fig. 2 we show that in 59 wells sampled a total of 66 B-cell clones were generated. If we now hypothesise that these B-cell clones were the result of a 1 T cell : B cell interaction then the clone numbers would be expected to segregate in the wells according to a Poisson distribution of T cells, as these are the cells which are randomly distributed. Table 1 shows the numbers of clones observed in each well compared to that which would be expected by a Poisson distribution of the total 66 clones, and it can be seen that the fit is very close ($\chi^2 = 1.76$). Thus the most accurate estimate we can derive from this data is that one T cell is only able to cooperate with a single hapten specific B cell. The number of wells containing multiple clones can be accounted for by chance distribution of T cells, however, we cannot exclude the possibility that a minority of the T-cell population could cooperate with more than one B cell.

This finding shows that observations which indicated marked restriction in the number of B cells helped by a single T cell were indeed the consequence of a monogamous behaviour of helper T cells. The discrepancies with data showing heterogeneity[3] may be the result of the nature of the antigen used since in situations where heterogeneous foci are detected, particulate antigens have been used and with particulate antigens T cells are able to produce a soluble factor (NSF) which can easily replace helper T cells.

The finding that one T cell only reacts with one B cell does not support the idea that this cooperation has occurred through specific factors unless the activity of these factors is short ranged. Either way, the data is not compatible with the concept of widely diffusible specific mediators[9,10].

An alternative explanation is that there may be subsets of B and T cells such that each T cell is able to react directly with or release factors which could react with only a certain proportion of the possible B cells. There is some evidence that individual T cells release NSF which is specific for a subpopulation of the B-cell repertoire[11] and it is possible that similar restrictions are placed on specific helper T cells. One situation where specific helper cells have been shown to be selective is in the case of allotype specificity[12]. The same selectivity may exist among T cells for immunoglobulin class[13,14].

If single T cells could be presented with a large enough range of B cells it should be possible to over-ride any 'matching' restrictions. In our experiments we can only be assured of 5–12 B cells per well and so far attempts to enrich the B cell population for trinitrophenol (TNP) specific B cells have only increased the numbers of hapten specific B cells by two- to fourfold and this did not affect the observed restriction. Enriching for hapten specific B cells would also rule out the possibility that the T cells are not able to 'find' more than a very near B cell.

The third possible explanation for the result is that cooperation between specific T and B cells requires cell-to-cell contact and that once this has taken place the T cell is then, somehow, rendered inactive towards other B cells. Should this in time be shown to be the correct explanation then it must be resolved just how two such rare antigen specific cells (T and B) could locate each other.

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Dissociation of effector functions in populations of activated macrophages

THERE is much evidence to indicate an important role for activated macrophages in resistance to malignant tumours and to infections caused by intracellular pathogens. Different investigators have found that activated macrophages may be distinguished from normal macrophages by either morphological, biochemical, or functional criteria, depending on the experimental design[1]. Functional criteria frequently cited are killing of intracellular organisms[2,3], inhibition and/or killing of tumour cells[4-6] and inhibition of proliferating lymphocytes[7,8]. Previous work in our laboratory has demonstrated that macrophages activated by several methods have always exhibited each of these three functional capacities. For example, macrophages activated by either chronic infection with C56 strain *Toxoplasma gondii* or by killed *Corynebacterium parvum* inhibited multiplication of *T. gondii*[9-11], DNA synthesis by tumour target cells[12,13], and mitogen- and antigen-specific lymphocyte transformation[14]. Whether different populations of activated macrophages which have been defined by one functional criterion always possess the other functional capacities of activated macrophages as well, however, has not been established. We report here that activated macrophages characterised by one functional criterion do not necessarily possess other functional characteristics of activated macrophages and that differences in functional capacity depend on the method used to activate the macrophages.

Three models for activating macrophages were used: *Trichinella spiralis* infection in C57Bl/6 mice (ref. 15 and E.J.W. and J.S.R., in preparation), C37 strain *T. gondii* infection in BALB/c mice[16] and C56 strain *T. gondii* infection in Swiss Webster mice[9]. Peritoneal exudate cells, used as a source of macrophages, were harvested and processed as previously described[17]. Macrophage monolayers were challenged with either El-4 tumour cells to test inhibition of tumour cell DNA synthesis[17] or RH strain *T. gondii* to test inhibition of intracellular multiplication[18].

Table 1 Tumour cell cytostasis and inhibition of intracellular multiplication of *T. gondii* by activated macrophages

Infection used to activate macrophages	Time after infection	Tumour cytostasis (CI)	Infected macrophages with multiplying *T. gondii* (%) Normal	Experimental	Activated
T. spiralis in C57B1/6 mice	2 weeks	98	96	98	0
C37 strain *T. gondii* in BALB/C mice	19 weeks	94	98	98	0
C56 strain *T. gondii* in Swiss Webster mice					
0 h incubation	16 weeks	99	98	0	—
24 h incubation	16 weeks	−14	99	1	—

Mice infected with *T. spiralis* were used 7–28 d after infection, those with C37 strain *T. gondii* infection at 19 weeks after infection, and those with C56 strain *T. gondii* infection 3–6 months after infection. Macrophages from the C56 strain *T. gondii*-infected mice were tested either immediately or after 24 h of *in vitro* incubation. To test tumour cell cytostasis by macrophages, 8 to 9×10^5 peritoneal exudate cells from appropriate mice were allowed to adhere for 3 h to 6-mm wells of 96-well microtitre plates (Flow Laboratories). The wells were then washed thoroughly with saline to remove nonadherent cells. E1-4 tumour cells (2×10^4 in 0.2 ml medium 199 with penicillin 100 IU ml^{-1}, streptomycin 100 μg ml^{-1}, and 10% heat-inactivated foetal calf serum) (M199-FCS) were added to each well. Incubation was carried out for a total of 24 h at 37 °C. Six hours before termination of the culture, the wells were pulsed with from 0.5 to 1.3 μCi (^{3}H)TdR (specific activity 6 Ci mmol^{-1}; Schwarz–Mann). The cells were collected with a MASH II harvester (Microbiological Associates) and then counted in a scintillation spectrophotometer. A cytostatic index (CI) was defined as $((N-A)/N)\times100$ where N is c.p.m. of tumour cells plus normal macrophages and A is c.p.m. of tumour cells plus activated macrophages. To measure inhibition of intracellular multiplication of *T. gondii* by macrophages, macrophage monolayers were prepared as previously described[17] and then challenged with 2×10^6 RH strain *T. gondii* trophozoites in M199–FCS for 1 h. The monolayers were washed and allowed to incubate in M199–FCS for 18–24 h. The slides were washed, fixed and stained with Giemsa stain. The percentage of infected cells with multiplying *T. gondii* was determined by light microscopy. Normal macrophages were from unimmunised mice; activated macrophages were from mice chronically infected with C56 strain *T. gondii.*

Experiments measuring the ability of macrophages to inhibit tumour cell DNA synthesis and to inhibit intracellular multiplication were run in parallel. In all experiments, macrophages from immunised mice were compared with both macrophages of normal mice (which do not inhibit DNA synthesis of El-4 tumour cells or inhibit intracellular multiplication of *T. gondii*) and macrophages from mice chronically infected with C56 strain *T. gondii* (which inhibit both).

The results are shown in Table 1. Macrophages from both *T. spiralis*-infected mice and C37 strain *T. gondii*-infected mice had the capacity to inhibit tumour cell DNA synthesis. These same macrophages were unable to prevent intracellular multiplication of *T. gondii*. For *T. spiralis*-infected mice, the dichotomy between the two functional capacities was observed in mice infected with from 200–530 larvae (significant mortality occurred above 530 larvae) and from 7 to 28 d after infection. For mice infected with C37 strain *T. gondii*, the dichotomy was observed at 19 weeks after infection.

Macrophages removed from mice infected with C56 strain *T. gondii* and challenged immediately inhibited both DNA synthesis by tumour cells and intracellular multiplication by *T. gondii*. After macrophages had incubated *in vitro* for 24 h, however, the capacity to inhibit DNA synthesis by tumour cells was lost, but the capacity to inhibit intracellular multiplication of *T. gondii* was retained. The density of the macrophage monolayers, which does affect tumour cytostasis, did not change significantly during *in vitro* incubation. Thus, macrophages in the first two experiments inhibited tumour cell DNA synthesis but not intracellular killing, whereas in the third experiment the converse was true.

Our results show that macrophages characterised as activated by one criterion may not satisfy other criteria of activation. That our observations were made in three unrelated experimental systems suggests that this phenomenon is common. The functional differences in different macrophage populations which we have observed may explain the variable results in previous studies and suggest that future publications that discuss activated macrophages should specifically state the criteria used to define the macrophages as activated.

Among the possible explanations for the variability in macrophage functions which we observed is the hypothesis that the functions of activated macrophages in a given population are identical at any one time. The functions of a given population, however, may vary with a number of factors, such as the nature of immunisation and the time after immunisation. Thus, for example, during an acute infection, when a maximum stimulus to activation occurs, all properties of activated macrophages may exist. These properties might be lost at different rates, and therefore at various times after infection only certain properties of activated macrophages are manifested.

A second hypothesis is that different subpopulations of effector macrophages with different functional capacities exist[19,20]. Thus, macrophages which inhibit tumour cell DNA synthesis may be distinct from those which inhibit intracellular multiplication. Our observation that in two experimental systems, inhibition of tumour cell DNA synthesis existed alone, whereas in the third inhibition of intracellular multiplication existed alone suggests this second possibility. The complete separation of functions we observed may be more consistent with the existence of different functional populations of activated macrophages.

The separation of the capacity of macrophages to inhibit multiplication by intracellular pathogens and to inhibit DNA synthesis by tumour cells should allow for more exact studies of the functions and biochemistry of activated macrophages.

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Antiviral T cell-mediated immunity in Marek's disease

MAREK'S disease (MD) is a herpesvirus induced lymphoma of chickens characterised by transient virus replication in lymphoid cells and transformation of T cells[1]. Several mechanisms probably operate in resistance to Marek's disease, but there is indirect evidence that protective immunity is predominantly T-cell dependent[2] and comprises both antiviral[3] and anti-tumour[4] immune responses. Thus, a notable feature which distinguishes immunity to Marek's disease from immunity to other herpesvirus infections studied so far is that both effector and target cells are probably T cells and that both virus and tumour-specific antigens may act as targets for immunological attack. Using a plaque inhibition test as a measure of cell-mediated immunity, I show here that T cells obtained from immunised birds are sensitised to virus-specific antigens present at the surface of infected cells of both lymphoid and non-lymphoid origin. The results provide direct evidence for the role of T cells in eliminating infected leukocytes and in reducing the spread of Marek's disease virus (MDV) *in vivo*.

Sensitised lymphocytes were obtained from an inbred line of White Leghorns (Line 7) immunised at three weeks of age by intraperitoneal inoculation of 10^3 plaque-forming units (PFU) of cell-associated attenuated MDV (HPRS-16/att)[5]. Lymphocytes were isolated from peripheral blood by flotation in Ficoll–Hypaque and were washed three times in phosphate-buffered saline before use in plaque inhibition tests. Of the cells obtained, 90%–97% were small lymphocytes and 1.5%–2.0% were monocytes. Two types of tests were carried out. (1) Sensitised cells and MDV-infected cells (target cells) were mixed in suspension and were plated on monolayers of kidney cells derived from Line 7 chickens. Monolayers were washed after 24 h and plaques allowed to develop. (2) Monolayers of kidney cells were pre-infected with cell-associated MDV for 24 h before addition of sensitised cells. The first test was intended to study direct killing of lymphoid and non-lymphoid target cells. The second was originally designed to study predominantly the effect of sensitised cells on cell to cell spread of virus in monolayers of non-lymphoid cells.

The kinetics of plaque survival with increasing concentration of sensitised cells were consistent with a one-hit process of inactivation. This was noted in both types of tests and for both lymphoid and non-lymphoid targets (Fig. 1). Similar results were obtained in several experiments using sensitised lymphocytes derived from birds 20–70 d after immunisation. These results strongly suggest that sensitised cells act independently in preventing plaque formation and that target antigens are found on both infected lymphoid cells and on infected kidney cells. It has consistently been found that plaque inhibition was 1.5 to 2-fold greater when leukocyte-associated MDV was used as targets as compared with infected kidney cells. The differences observed in both types of tests using sensitised lymphocytes obtained 20–90 d after immunisation were significant ($P<0.01$ (seven experiments), Student's t test). The reason for this difference in susceptibility is not known, but it is possible that kidney-cell associated virus adapted to growth *in vitro* initiates infection more rapidly than leukocytes freshly isolated from infected birds. In this instance, it is known that the spread of MDV from infected leukocyte to kidney cells is delayed in at least a proportion of the cells and it is possible that the probability of plaque inhibition is greater for these cells

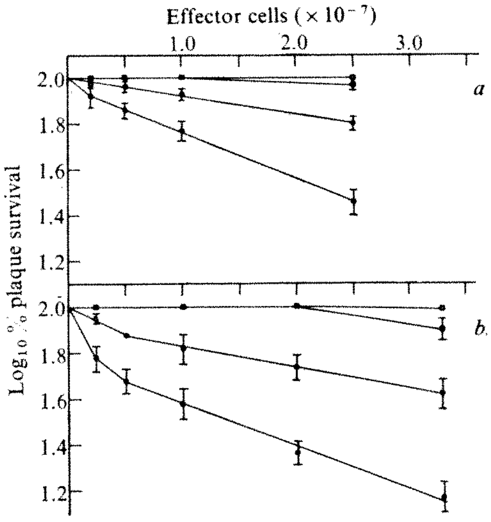

Fig. 1 Kinetics of plaque inhibition. *a*, Aliquots of 10^7 leukocytes obtained from Line 7 birds naturally infected with MDV or of 500 PFU of cell-associated MDV propagated in kidney cells derived from Line 7 birds were mixed in suspension with different numbers of sensitised leukocytes obtained from syngeneic birds. The mixture was plated for infective centres in duplicate on monolayers of syngeneic kidney cells grown in 50-mm Petri dishes. After 24 h at 37 °C, monolayers were washed and fresh medium added. Plaques were counted after 5 d. In this experiment, sensitised lymphocytes were obtained 50 d after immunisation. Lymphocytes used as controls to sensitised lymphocytes were obtained from normal Line 7 birds of identical age. Results shown are means of four estimations using lymphocytes obtained from different birds for each estimation, and are expressed as plaque survival relative to untreated cultures. Bar represents s.e.m. ●, Sensitised lymphocytes + leukocyte-associated MDV targets. ○, Sensitised lymphocytes + kidney cell-associated MDV targets. ■, Normal lymphocytes + leukocyte-associated MDV targets. □, Normal lymphocytes + kidney cell-associated MDV targets. *b*, Monolayers of Line 7 kidney cells in 50 mm Petri dishes were preinfected with leukocyte-associated MDV or with kidney cell-associated MDV. Medium was replaced after 24 h and sensitised or normal lymphocytes added. In this experiment, sensitised lymphocytes were obtained 30 d after immunisation. Cultures were refed after 48 h and plaques were counted 3 d later. Symbols as for Fig. 1*a*.

than for those which allow more rapid transfer of infectious virus. Thus, the survival curve obtained using monolayers pre-infected with leukocyte-associated MDV (Fig. 1*b*) probably reflects the survival of at least two cell populations differing in susceptibility to killing, each being inactivated with one-hit kinetics.

Although there was evidence of nonspecific inhibition using high concentrations of normal lymphocytes, the inhibitory effect observed at lower concentrations of sensitised cells was virus-specific. Thus, provided that the number of sensitised cells added to 50-mm dishes did not exceed 3×10^7, plaques formed by MDV only were inhibited. Vaccinia and herpes simplex viruses were not affected. Further evidence for virus specificity was obtained by absorption experiments. For this purpose, sensitised cells were cultured on fixed monolayers of MDV-infected kidney cells or of normal cells. Lymphocytes recovered from normal cell monolayers were still active in plaque inhibition tests whereas those recovered from MDV-infected monolayers were no longer capable of inhibiting plaques (Table 1).

Since sensitised cells were removed by absorption to infected but not to uninfected kidney cell monolayers, it is concluded that plaque inhibition is virus-specific. It follows also that the cells which participate in plaque inhibition are normally non-adherent and that they comprise not more than 1–2% of the population of sensitised cells (Table 1).

The antigens which interact with sensitised cells have not been characterised here, but it is likely that at least some of them might be hitherto unrecognised antigens not detectable using convalescent Marek's disease serum. This follows indirectly from the fact that although Marek's disease serum reacts with antigens at the surface of MDV-infected kidney cells in immunofluorescence tests (17% were positive, Fig. 1*a*), it does not react with infected leukocytes or with infected leukocytes which have been cultured for 24 h. Yet, infected leukocytes could be prevented from forming plaques after a relatively short time of contact with sensitised cells, and the inhibitory effect was abolished by absorbing sensitised cells to MDV-infected kidney cells. It is possible that some of the antigens which act as targets on MDV-infected leukocytes are analogous to cell surface antigens reported in non-producer EBV positive cell lines of Burkitt's lymphoma[6]. Such cells could be lysed by sensitised T lymphocytes although they did not express serologically detectable cell surface antigens.

To determine the nature of the effector cells which operate in plaque inhibition, sensitised cells were pretreated with antisera specific for chicken T and B cells, and their effect on plaque formation estimated. Significant reduction in the capacity of sensitised cells to inhibit plaques was noted only when they were pretreated with anti-T serum and complement (Table 2). As the anti-T and anti-B sera have been shown to be cytotoxic predominantly for homologous cells by the trypan blue exclusion test, it is concluded that plaque inhibition is mediated by T cells.

Table 2 Effect of anti-T serum on the capacity of sensitised cells to inhibit plaque formation

Treatment	Experiment 1 Monolayer infected with kidney cell-associated MDV	Experiment 2 Monolayer infected with leukocyte-associated MDV
Sensitised lymphocytes		
+anti-T+complement	80±3.2*	150±20.1*
+complement	61±2.3	69± 3.0
+normal rabbit serum +complement	65±5.1	75±4.0
+anti-B+complement	54±3.7	91±10.4
+anti-chick Ig +complement	68±4.3	75± 8.3
+anti-T+anti-chick Ig +complement	85±2.6*	141.5±14.9*
Sensitised lymphocytes	60±5	70±8.2
Untreated	80±4.6	160±10.2

Sensitised lymphocytes obtained 50 d after immunisation were treated at a concentration of 5×10^7 per ml with antiserum and complement[10]. Antisera against chicken thymus cells (anti-T) and against chicken bursal cells (anti-B) were obtained from rabbits immunised with T and B cells[11]. Anti-chick Ig was obtained commercially (Nordic). All antisera were used at a final dilution of 1/10. Guinea pig serum absorbed to agarose was used at a final dilution of 1/10 as a source of complement. Treated and untreated sensitised cells were washed twice in RPMI medium +10% foetal calf serum before use in plaque inhibition tests. These were carried out as described for Fig. 1*b* with the exception that monolayers were grown in 30-mm dishes and 3×10^6 sensitised cells were used for each dish. Values (means±s.e.) of plaque counts using lymphocytes from four different birds. Analysis of variance using orthogonal contrast to find the difference between treatment means showed that only treatment with anti-T serum abrogated the capacity of sensitised cells to inhibit plaques.

$*P < 0.001$.

The mechanism of plaque inhibition reported here is different from that reported by others for herpes simplex[7], infectious bovine rhinotracheitis[8] and for varicella–zoster[9] viruses. For these cases, plaque inhibition was attributed to interferon released either as a result of interaction between sensitised peritoneal macrophages and antigen or between sensitised lymphocytes, monocytes and antigen. I was unable to detect interferon-like substances in the medium obtained from cultures in which inhibition of MDV plaques had occurred. Plaque formation by herpes simplex

Table 1 Specificity of plaque inhibition: effect of absorbing sensitised cells to MDV-infected monolayers

	Experiment 1				Experiment 2			
Effector cells	Absorbed to normal cells		Absorbed to MDV-infected cells		Absorbed to normal cells		Absorbed to MDV-infected cells	
Control	140±10	*	124±4	NS	281±9	*	269±19	NS
MDV-sensitised	72±3		128±10		189±4.5		237±17	

Monolayers of normal kidney cells derived from Line 7 birds and of cells heavily infected with kidney cell-associated MDV were fixed with 0.1% glutaraldehyde in phosphate-buffered saline (PBS) for 30 min at room remperature and were thoroughly washed with PBS. Approximately 5×10^7 viable lymphocytes obtained from control birds or from immunised birds 40 d after immunisation were added to the monolayers in 50-mm Petri dishes and were incubated for 3 d at 37 °C in RPMI medium (Flow Laboratories)+10% foetal calf serum. Non-adherent lymphocytes obtained from the medium and by washing the monolayers once were pooled, washed, and used for plaque inhibition. The proportions of the original number of viable lymphocytes plated recovered from monolayers of normal cells were: 7% (control) and 7.2% (MDV-sensitised). Corresponding values for recovery from MDV-infected monolayers were: 6% (control) and 4.8% (MDV-sensitised). Differences in recovery were not significant except in the case of MDV-sensitised lymphocytes cultured on MDV-infected monolayers (the mean reduction in recovery of 1.2% compared with absorption to normal cells was significant, $P < 0.05$ for both experiments). Plaque inhibition tests were carried out as described for Fig. 1 using 5×10^6 sensitised or control lymphocytes as effector cells. Targets in experiment 1 were monolayers preinfected with leucocyte-associated MDV. In experiment 2, leukocyte-associated MDV targets were mixed with effector cells and were plated for infective centres as described for Fig. 1*a*. Values are means±s.e. of plaque counts of four estimations using lymphocytes from four birds for each treatment.

$*0.01 < P < 0.05$.

NS, not significant (Student's *t* test).

was not inhibited when herpes simplex-infected cells, MDV-infected cells and MDV-sensitised lymphocytes were plated simultaneously for infective centres. Similar results were obtained using vaccinia-infected cells (unpublished). Moreover, the cells which participate in plaque inhibition are non-adherent and therefore not macrophages.

Several conclusions can be drawn from these experiments. First, sensitised T cells act independently to inhibit plaque formation. Second, inhibition is probably due to direct lethal interaction between sensitised cells and MDV-infected target cells. Third, hitherto unrecognised virus-specific antigens present at the surface of infected leukocytes may act as targets for sensitised lymphocytes. The plaque inhibition test reported here should prove a better *in vitro* measure of protective immunity than conventional tests for cell-mediated immunity since there is evidence that leukocyte-associated viraemia is invariably reduced in immunised birds compared with controls on infection with virulent MDV (refs 3, 12). A mechanism similar to that reported here for plaque reduction may thus operate *in vivo* to eliminate infected leukocytes and the spread of MDV throughout the tissues of infected birds.

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Heterospecific cytotoxic cell activity induced during the first three days of acute lymphocytic choriomeningitis virus infection in mice

ACUTE infection of adult mice with lymphocytic choriomeningitis virus (LCMV) induces several types of immune responses. H-2-restricted virus-specific cytotoxic T cells are demonstratable from day 5 to 6 and peak on day 7 to 10 post-infection[1–5]. Antiviral antibody in immune complexes is first detected by 4 d post infection[6,7], and non-specifically activated macrophages can be demonstrated after 4 to 5 d (refs 3 and 8). We report here that the acute LCMV infection elicits the production or activation of another type of cytotoxic cell as early as 1 d post infection. These early cytotoxic cells lyse infected or uninfected syngenetic, allogeneic, and xenogeneic cell lines with some specificity. These effector cells do not belong to the cell types thus far implicated in the immune response to LCMV infection and have many of the properties of natural killer cells.

C3H/St (H-2^k) mice were infected intraperitoneally with 2×10^4 plaque forming units (PFU) of the Armstrong strain of LCMV at various days. Their spleens were tested in one test for spleen cell killing of uninfected or LCMV-infected L-929 cells (H-2^k). Figure 1 shows that as early as one day post infection and peaking on the third day post-infection, spleen cells killed uninfected and infected L cell targets with equal efficiency. By the fifth and sixth day post infection, the virus-infected cells were lysed with much greater efficiency than uninfected cells, probably by cytotoxic T cells. During the first 4 d post infection, comparable killing efficiency on a cell-to-cell basis was obtained when peritoneal cells were used as the effector cells. Most subsequent experiments used cytotoxic cells isolated from the spleen or peritoneal fluid of mice at 3 d post infection. Exposure of the LCMV preparation to ultraviolet irradiation[9] before infection of mice abolished its ability to induce the early cytotoxic cells, and the culture fluid of L cells (used to propagate LCMV) did not elicit the killer cell response. Although most ^{51}Cr release assays were run for 16 h, slightly more than 50% of the killing activity occurred during the first 4 h (Table 1*a*). No early cytotoxic cell killing was observed if spleen cells were first incubated overnight at 37 °C before exposure to labelled targets (Table 1*b*). In contrast, the later virus-specific day 8–10 T-cell killing was not reduced by overnight incubation, indicating that the early cytotoxic cells were less stable to *in vitro* incubation than T cells.

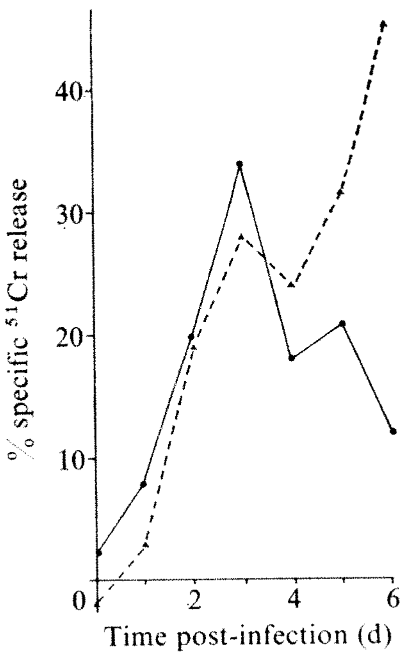

Fig. 1 C3H/St mice were infected intraperitoneally with 2×10^4 PFU of Armstrong strain LCMV. At daily intervals post infection the spleens were collected and cells liberated by gentle squeezing with forceps. Erythrocytes were lysed by exposure to 0.83% NH_4Cl. The erythrocyte-depleted spleen cells were then exposed to 10^4 ^{51}Cr (New England Nuclear) labelled L cells, either uninfected or persistently infected with LCMV[23]. In a total volume of 200 µl in microtitre wells (Falcon no. 3040), spleen cells were added at various effector-to-target ratios (100: 1, 50: 1, 25: 1). Data expressed are at effector-target ratios of 100: 1. Samples were incubated for 16 h before collecting cells. Background release of ^{51}Cr was that obtained with cells incubated under Eagle's minimal essential medium (MEM). Maximum lysis was determined by exposing labelled cells to 1% NP40. Per cent specific ^{51}Cr release was calculated as

$$\frac{(\text{c.p.m. test sample}-\text{c.p.m. MEM control})}{(\text{c.p.m. NP40 control}-\text{c.p.m. MEM control})}\times100$$

All assays were run in quintuplicate, and the standard error of this assay method was < 5% of the mean. The zero time samples represent spleen cell killing from uninfected mice. The values given here and on subsequent tables represent the average of the mean. ●, L cell target; ▲, L(LCMV) cell targets.

The early cytotoxic cells killed a wide variety of cultured syngeneic, allogeneic, and xenogeneic cells. Usually 15–45% of the target cells were killed within 16 h at effector-to-target cell ratios of 100: 1. Spleen cells from

C3H/St (H-2^{k}) mice killed L-929 (H-2^{k}), EL-4 (H-2^{b}), P815 (H-2^{d}), J774 (H-2^{d}), and SCRF 179 lymphoblast (H-2^{z}) mouse cell lines, as well as F-9 cells, which have no detectable H-2 antigens[10]. They also killed continuous primate cell lines (HeLa, Raji, Wil-2, and Vero), as well as early passage mouse (C3H/St and NIH Swiss), rat (BN and Lewis), hamster (Syrian)and human fibroblasts. In contrast, they did not kill freshly harvested unstimulated peritoneal macrophages or splenic lymphocytes from C3H/St mice. The ability of the early cytotoxic cells to kill such a heterogeneous variety of cultured cells suggested nonspecificity, but cold target blocking experiments indicated some degree of specificity (Table 2). Three mouse cell lines with different H-2 specificities and one human cell line (Raji) were exposed to the early cytotoxic cells, and all were lysed at significant levels above controls. Unlabelled cells of each type were then added to the assay wells in sixfold excess of the labelled targets. In all combinations, unlabelled target cells competed best with labelled homologous targets. Nonhomologous cells partially blocked the cytotoxicity to various degrees, suggesting some shared target cell specificity.

Table 1 Stability of early cytotoxic cells in culture % specific ^{51}Cr release

Experiment *a* Duration of assay on L cells (h)

	1	4	8	12	24
Day 3 effector cells	4.5	18	22	21	23
Control effector cells	−1.0	−1.5	3.0	−0.5	2.5

Experiment *b* Cytotoxic activity following incubation in culture

Time of assay after collecting spleen cells (h)	Target	Day of spleen cell harvest 2	4	8	10
0–16	L	16	21	8.8	−0.5
22–38	L	−2.4	−3.4	8.5	1.0
0–16	L(LCMV)	21	29	50	29
22–38	L(LCMV)	−1.3	0.0	64	30

Cells from C3H/St mice taken at the designated times after infection were tested at effector–target ratios of 100 for cytotoxic activity either immediately after harvest (experiment *a*, *b* 0–16), or after overnight incubation (experiment *b* 22–38).

Early cytotoxic cells were detected after acute LCMV infection in both male and female mice of the following strains: AKR, BALB/c, B10.Br, B10.D$_2$, CBA/N × DBA/2 F$_1$, C3H/HeJ, C3H/St, C3H/St × DBA/2 F$_1$, NZB × NZW F$_1$ and athymic nude (BALB/c) mice. The latter result with nude mice suggested that the early cytotoxic cell was not a T cell, and this was confirmed by our findings from four experiments that depletion of spleen T cells with antibody to θ antigen plus complement failed to remove the cytolytic activity (Table 3*a*). Whereas the cytolytic activity of day 8 (T) cells on LCMV-infected syngenetic targets was reduced by 78%, the activity of the day 3 early cytotoxic cells on uninfected targets was enhanced.

Table 2 Specificity of early cytotoxic cell % specific ^{51}Cr release

Effector cell	Blocking cell	Targets EL4*	L929	P815	Raji
Control	None	17	0	1.5	1.5
LCMV	None	44	19	17	25
LCMV	EL4	10	13	11	7.5
LCMV	L929	39	2.6	7.6	16
LCMV	P815	30	15	3.0	16
LCMV	Raji	30	8.1	7.6	1.5

Early cytotoxic spleen cells from C3H/St mice were exposed to ^{51}Cr labelled cell targets at an effector to target ratio of 100. Unlabelled target cells in sixfold excess of the labelled targets were incubated with the labelled targets in some of the reactions (blocking cell). Spleen cells from uninfected control mice were also tested for their cytolytic activity.

*These EL-4 cells express oncornavirus antigens on their cytoplasmic membranes.

Further analyses indicated that the early cytotoxic cell was neither a macrophage nor a polymorphonuclear leukocyte (PML). Most of the cytolytic activity of peritoneal cells isolated from either C3H/St or nude (BALB/c) mice was associated with the non-adherent population (Table 3*b*) after adsorption to plastic. In three other similar adsorption experiments with spleen cells, the killing activity on a cell-to-cell basis was enriched by 5–25% following removal of adherent cells. When macrophages and PML were removed by passing spleen cells through a glass wool column, the effluent cells, which contained <1% macrophages and <2% PML, retained all of their cytolytic activtiy.

We found that during the first 3 d after LCMV infection in adult mice, cytotoxic cells appear that kill many types of uninfected or infected cultured cells with some specificity but do not kill primary macrophages or lymphocytes. Pfizenmaier *et al.*[4,5] have reported that 5 d after infection of mice with LCMV, cytotoxic T cells are generated which kill both infected and uninfected syngeneic targets, and Blanden and Gardner[11] have described the appearance of early killer T cells that lysed uninfected syngeneic targets 2–4 d after infection of mice with ectromelia virus. Rodda and White[12] reported a possibly similar early killer cell during Semliki Forest virus infection of mice but concluded that it was a macrophage. The early cytotoxic cell that we describe here is clearly neither a T cell nor a macrophage nor a PML. Cytotoxic cells which are labile at 37 °C and kill tumour cells but not primary cell lines have been reported and designated natural killer (NK) cells. These cells lack surface antigen markers (IgG, Fc receptors, C3 receptors, θ antigen) and lyse Moloney leukaemia/sarcoma virus infected or transformed cell targets[13-17]. The LCMV infection may activate normally undetectable NK cells that have some additional target specificities. A similar induction of NK cell activity in mice can be elicited by Bacille Calmette-Guerin (BCG) (ref. 18). An alternative hypothesis is that the LCMV infection may induce a polyclonal natural antibody response which would mediate antibody-dependent cell cytotoxicity (ADCC) by K cells, cytolytic lymphocytes with Fc receptors[19,20].

Table 3 Characterisation of early cytotoxic cells % specific ^{51}Cr release

Experiment *a* Depletion of spleen T cells*

Effector cell	Target cell	Treatment of effector cell None	Anti-θ + C	C
Day 3	L	10	13	9.3
Day 8	L(LCMV)	63	14	62

Experiment *b* Adherence properties of peritoneal killer cells†

Effector cell	C3H/St mouse	Nude (BALB/c) mouse
Day 3 non-adherent	17.4	22.2
Day 3 adherent	5.5	5.8
Day 3 unseparated	18.5	26.6
Control unseparated	0.4	0.5

*Spleen cells from days 3 or 8 post infection were treated with antibody to θ antigen (a gift from R. Blanden) and guinea pig complement (C), which had previously been absorbed against C3H/St mouse spleen cells. After incubation, the spleen cells were washed and tested on labelled L or L(LCMV) targets at an effector–target ratio of 50.

†Peritoneal cells were adhered to plastic microtitre wells for 2 h at a concentration of 5×10^{5} cells per well (effector–target ratio = 50). The non-adherent cells were gently pipetted off the monolayers and transferred to other wells without adjusting the cell number. A monolayer of adherent cells (> 80% macrophages) was left. Target L cells were added to the wells for a ^{51}Cr release assay.

Epstein–Barr virus has been reported to induce polyclonal B-cell activation of cultured human lymphotcytes[21]. We have been unable to establish a functional role for immunoglobulins or Fc receptors in our system, and little activity is associated with populations of adherent cells (Table 3), known to be effectors of ADCC in mice[22]. This phenomenon cannot be explained by the transfer of virus with the spleen cells to the labelled targets, rendering them sensitive to anti-LCMV ADCC because (1) there is some target cell specificity, (2) most killing occurs at a time (4 h) before the viral antigens could be expressed on the surface of infected target cells, (3) Raji cells are sensitive targets yet refractory to LCMV infection (R.M.W., unpublished), and (4) very little virus infectivity is transferred in macrophage depleted spleen cell populations. Thus, although we cannot yet completely exclude an ADCC mechanism, our data describing a heat labile, non adherent, non-T lymphocyte active against a variety of cell targets in the absence of added antibody suggest that the early cytotoxic cell generated during the first few days of the LCMV infection is similar to an NK cell.

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Note added in proof: Inductions of NK-like cells in mice by acute virus infections have recently been observed by several other laboratories[24–26].

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Protein covalently linked to foot-and-mouth disease virus RNA

THE RNA in picornaviruses can function directly as mRNA (ref. 1). In contrast to the great majority of cellular and virus mRNA molecules, however, a 'cap' structure has not been found at the 5′-end of the RNAs of polio[2,3] and encephalomyocarditis (EMC) (ref. 4) virus particles or on poliovirus specific mRNA in infected cells[2,3]. The 5′-terminus of poliovirus mRNA extracted from polyribosomes was found to be pUp[2,3] but no nucleotide could be identified at the 5′-end of the RNA in virus particles. This is because a small protein is covalently linked to the 5′-terminal nucleotide of the RNA (refs 5, 6). The RNAs of EMC virus and foot-and-mouth disease virus (FMDV) contain a tract of about 100–200 cytidylic acid residues located near the 5′-end[7,8] but such a sequence was not found in poliovirus RNA (ref. 9). It was of interest, therefore, to determine whether a protein is also covalently linked to FMDV RNA and whether it is attached to the poly C tract. We show here that FMDV RNA is not capped and demonstrate the presence of a protein of molecular weight (MW) about 4,000 covalently linked to the RNA. The protein is not attached directly to the poly C tract but is probably attached to the 5′-terminus and can be removed without loss of infectivity.

When FMDV particles labelled with ^{3}H-uridine and ^{35}S-methionine were extracted with phenol–chloroform in the presence of sodium dodecyl sulphate (SDS), about 0.03% of the ^{35}S counts remained in the aqueous phase together with all the ^{3}H-labelled RNA and could not be removed by repeated extractions with phenol–chloroform. After sedimentation through a sucrose density gradient containing 0.1% SDS, the ^{35}S counts coincided with the ^{3}H-labelled peak of RNA (Fig. 1*a*). The ^{35}S counts were still associated with the RNA after heating at 70 °C for 1 min in 100% formamide (Fig. 1*b*), or at 100 °C for 1 min in 5% SDS, 5% mercaptoethanol and 2.5 M urea (Fig. 1*c*), conditions which would be expected to disrupt non-covalent bonds between protein and RNA. The altered sedimentation profile of the RNA after heating at 100 °C (Fig. 1*c*) is probably due to non-random degradation.

In contrast, treatment with Pronase in the presence of 0.1% SDS completely removed the ^{35}S-labelled material from the RNA without altering its sedimentation profile (Fig. 1*d*). These results strongly suggest that the ^{35}S-labelled material is covalently attached to the RNA and is a protein. No ^{35}S-labelled material was associated with the RNA of virus grown in pre-labelled cells, suggesting that the protein is not host specific. Further evidence that the protein and RNA are linked covalently was obtained by digestion with a mixture of T_1 and pancreatic ribonucleases. After this treatment the ^{35}S material remained at the top of a sucrose density gradient demonstrating that it was not fortuitously sedimenting at a rate similar to that of the RNA. The protein is not required for infectivity since an ^{35}S-methionine labelled FMDV RNA preparation with a titre of 1.7×10^6 placque-forming units per ml and containing 266 c.p.m. per ml had the same titre and sedimentation rate but contained less than 7 c.p,m, per ml after Pronase treatment. This result is in accord with the observation that poliovirus particle and mRNAs have similar specific infectivities despite the lack of a 5′-linked protein on the mRNA (ref. 15).

To characterise the protein the RNA isolated from ^{35}S-methionine labelled FMDV was purified by sucrose gradient centrifugation and then analysed by electrophoresis on 20% polyacrylamide gels[11] with or without digestion with a mixture of T_1, T_2 and pancreatic ribonucleases. All the ^{35}S counts in the untreated material remained at the top of the gel but after digestion with the ribonucleases the ^{35}S-labelled material migrated as a band with an apparent MW of about 4,000. Co-electrophoresis of the FMDV RNA-linked protein labelled with ^{35}S-methionine and the corresponding protein from poliovirus labelled with ^{3}H-lysine on high cross-linked polyacrylamide gels[12] showed that they had similar molecular weights.

The 5′-end of the virus RNA was shown to be blocked by analysis of the nucleotides produced by digestion of ^{32}P-labelled RNA with a mixture of T_1, T_2 and pancreatic

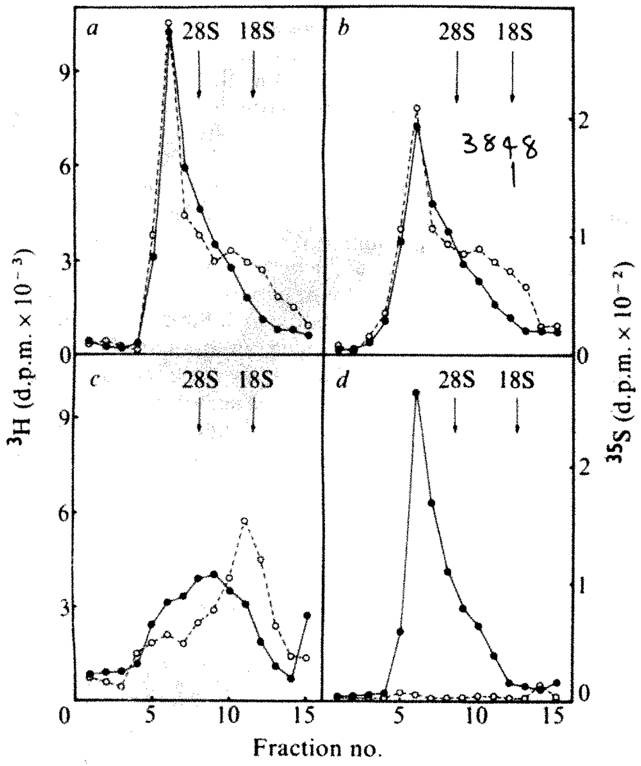

Fig. 1 Effect of denaturing conditions and Pronase on the sedimentation of FMDV RNA. The virus, type A61, was grown in the presence of ^{3}H-uridine (0.2 μ Ci ml^{-1}) and ^{35}S-methionine (25 μ Ci ml^{-1}, Radiochemical Centre, Amersham) and purified by sucrose gradient centrifugation[10]. The RNA was extracted with phenol–chloroform–SDS and precipitated at −20 °C with ethanol. Aliquots of the precipitated RNA were dried under N_2 and treated as follows before centrifuging at 50,000*g* for 16 h at 20 °C through 5–25% sucrose gradients in 0.1 M acetate –0.1% SDS, *p*H 5.0. The RNA was dissolved in *a*, 0.5 ml TNE–SDS (0.1 M Tris, 0.1 M NaCl, 0.001 M EDTA, 0.1% SDS, *p*H 7.5) and heated at 37 °C for 1 h; *b*, 0.1 ml 100% formamide and heated at 70 °C for 1 min before diluting to 0.5 ml with TNE–SDS; *c*, 0.1 ml disrupting solution (2.5 M urea, 5% mercaptoethanol, 5% SDS) and heated at 100 °C for 1 min before diluting to 0.5 ml with TNE–SDS; *d*, 0.5 ml TNE–SDS containing 50 μg Pronase and heated at 37 °C for 1 h. BHK ribosomal RNA was then added to each sample to serve as marker before centrifugation. Each fraction was precipitated with 10% TCA in the presence of bovine serum albumin (BSA) and filtered on to a glass fibre disk before counting in a liquid scintillation counter. ●, ^{3}H; ○, ^{35}S.

ribonucleases. Chromatography of the digest on DEAE–Sephadex in the presence of 7 M urea showed that there were no nucleotides eluting with a net charge expected for a nucleotide tetraphosphate (−6) or a capping group (−5 to −6) or a structure of the type pXp (−4) or ppXp (−5). This result is consistent with the presence of a free OH or a blocked structure at the 5′-end of the RNA in virus particles.

Further evidence that the 5′-end of the virus RNA was blocked was provided by electrophoresis on Whatman 3MM paper at *p*H 3.5 of the nucleotides produced by complete ribonuclease digestion. Four mononucleotide peaks were obtained together with small amounts of inorganic phosphate and cyclic mononucleotides, but in addition approximately 0.016% of the ^{32}P migrated towards the cathode. In contrast, less than 0.006% of the ^{32}P moved towards the cathode when the RNA was treated with Pronase before ribonuclease digestion. This suggests that a protein is attached to the 5′-end of FMDV-RNA in the same way that a protein is linked to poliovirus RNA[6].

As our previous work[8] had shown that the polyC tract in FMDV RNA is located near the 5′-end it seemed possible that the protein was attached to the polyC tract. To test this possibility the RNA prepared from FMDV labelled with ^{35}S-methionine and ^{3}H-cytidine was digested with ribonuclease T_1 and the oligonucleotide products separated by electrophoresis on a 12.5% polyacrylamide gel in 6 M urea at *p*H 8.5. The ^{35}S-methionine peak did not coincide with the ^{3}H-cytidine labelled polyC tract (Fig 2*a*) indicating that the protein was not attached to it directly. After T_1 and pancreatic ribonuclease digestion of ^{35}S-methionine-labelled RNA and subsequent electrophoresis on a similar gel all the radioactivity was found near the top of the gel (Fig. 2*b*), indicating that the protein does not move to the position shown in Fig. 2*a* because of its intrinsic size and charge at *p*H 8.5.

An important feature of the replication of FMDV is that less than 20% of the single stranded (ss) RNA synthesised in infected cells is encapsulated into virus particles[13]. Furthermore, the intracellular FMDV specific ssRNA sediments as a broad band in sucrose gradients with a peak at about 37S, whereas RNA extracted from virus particles sediments at 35S. The amount of protein associated with intracellular FMDV induced ssRNA was found to alter during the growth cycle. Only low levels of ^{35}S labelling were found on RNA up to the time when about half of the total RNA had been synthesised. Thereafter a greater proportion of ^{35}S labelling was observed and this continued to increase after RNA synthesis had virtually ceased. The

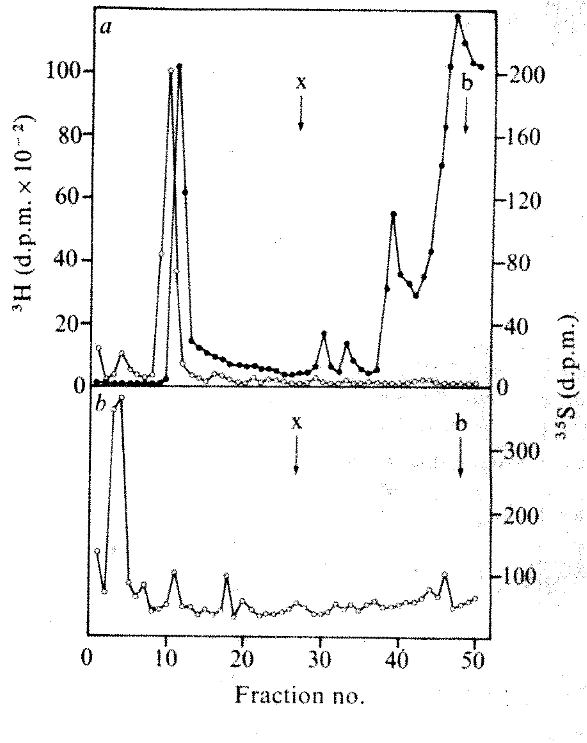

Fig. 2 Polyacrylamide gel electrophoresis of FMDV RNA after digestion with ribonuclease T1 or a mixture of T1 and pancreatic ribonucleases: *a*, Separate preparations of FMDV RNA labelled with either ^{3}H-cytidine or ^{35}S-methionine were prepared as described under Fig. 1 and purified further by centrifugation on 5–25% sucrose gradients in 0.1 M acetate–0.1% SDS, *p*H 5.0 at 20 °C for 18 h at 50,000*g*. The fractions containing the peak of RNA at about 35S were precipitated with ethanol in the presence of 100 μg *Escherichia coli* tRNA as carrier and digested with 30 units of ribonuclease T1 in 20 μl 0.01 M Tris-HCl 0.001 M EDTA containing 10 μg BSA for 1 h at 37 °C. *b*, 25 μg pancreatic ribonuclease added to the digestion mixture. The digested samples were mixed with an equal volume of dye marker (0.2% xylene cyanol (x), 0.2% bromophenol blue (b) in 50% sucrose, 6 M urea) and loaded on to 10 × 0.6-cm cylindrical 12.5% polyacrylamide gels made in 0.1 M Tris-borate, 0.0025 M EDTA, 6.0 M urea, *p*H 8.3 and subjected to electrophoresis for about 3 h at 100–150 V when the bromophenol blue had migrated 9 cm. The gels were sliced into 1.5-mm segments, digested with 0.5 ml NCS and the radioactivity determined by liquid scintillation counting in a toluene-based scintillation fluid, using appropriate spill-over corrections. ●, ^{3}H; ○, ^{35}S.

protein was associated with the entire heterogeneous induced ssRNA peak and not just with the 35S virus particle RNA.

We conclude from these observations that the ^{35}S-labelled material associated with the RNA extracted from ^{35}S-methionine-labelled FMDV particles is a protein of MW about 4,000 and is attached covalently. Our results are similar to those reported for poliovirus RNA (refs 5, 6). The protein linked to the RNA of poliovirus does not contain methionine[5] but that attached to the RNA of FMDV contains at least one methionine residue. If it is assumed that the protein in FMDV contains only one residue of methionine then the proportion of radioactivity in virus particles associated with the RNA-linked protein is compatible with the presence of a single copy in each virus particle.

The absence of a cap structure or an identifiable 5′ end on FMDV-RNA supports the hypothesis that, as with poliovirus RNA, a protein blocks the 5′ end of the RNA. The fact that the protein does not seem to be attached to the polyC tract suggests that this homopolymer is not 5′-terminal. The association of the protein with ssRNA parallels the appearance of mature virus particles in the cells and it is tempting to speculate that the protein has a regulatory role in determining whether the RNA is incorporated into virus particles as Fernandez-Munoz and Lavi have suggested for poliovirus[14]. The covalently-linked protein of poliovirus RNA has been found in replicative intermediate RNA (ref. 6) and on the 5′ end of negative RNA strands[15], leading to the suggestion that the protein has a role in transcription, acting as a primer on the nascent RNA strands and being cleaved from the RNA destined to become polysomal mRNA. A more detailed analysis of the fate of the RNA synthesised at different times after infection and an examination of the protein linked to the replicative intermediate should help to elucidate the role of the protein in virus replication.

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Participation of DNA-dependent RNA polymerase II in replication of influenza viruses

FOR many years it has been known that inhibitors of the expression of cell DNA, such as actinomycin D, prevent the multiplication of influenza viruses; inhibitors of DNA synthesis have no such effect[1–3]. This phenomenon is still not understood, even though from the outset the available data have suggested that cell gene transcription may be a necessary prerequisite for virus RNA transcription and replication[2]. The discovery that α-amanitin, a specific inhibitor of nucleoplasmic DNA-dependent RNA polymerase II (ref. 4), also specifically inhibits influenza virus replication[5,6], is additional support for the theory that cell gene expression is essential for virus growth. There has always been the possibility, however, that inhibition of virus growth is unrelated to the defined activities of DNA function inhibitors. It is possible, for example, that actinomycin D or α-amanitin might prevent the uptake or uncoating of influenza viruses, might inhibit directly the virion-associated transcriptase *in vivo*, or might act in some other unrecognised way. We describe here experiments in which α-amanitin was tested for its ability to inhibit the growth of influenza virus in cells containing an α-amanitin-resistant DNA-dependent RNA polymerase; in these circumstances, the virus grows normally in the presence of high concentrations of α-amanitin. This finding indicates that DNA-dependent RNA polymerase II has an essential role in the replication of influenza viruses.

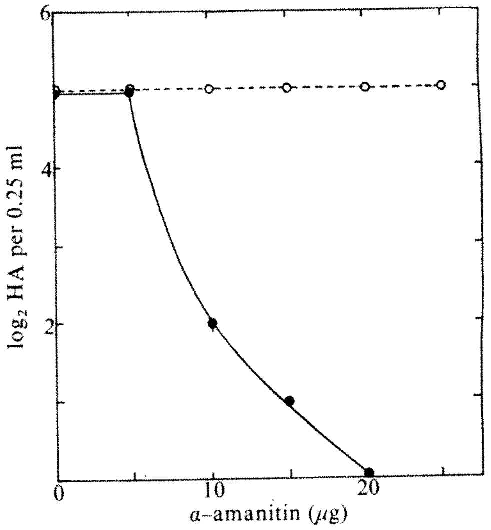

Fig. 1 Effect of increasing doses of α-amanitin on the yield of FPV from α-amanitin-sensitive, wild-type (●) and α-amanitin-resistant, mutant AMA-1 (○) CHO cells. Confluent monolayer cultures of cells were pre-incubated in medium containing α-amanitin for 1 h before infection with FPV (10 p.f.u. per cell), and α-amanitin was present in the culture medium throughout the experiment. At 24 h the amount of virus released into the medium was determined using an HA assay. Similar results were obtained when infected cells were disintegrated by Dounce homogenisation before HA determination.

The experiments were carried out on Chinese hamster ovary (CHO) cells infected with the avian influenza A virus, fowl plague (FPV). Two cell lines were kindly provided by Dr L. Siminovitch, University of Toronto, Canada, comprising the normal, α-amanitin-sensitive, wild-type CHO cell, and the mutant, α-amanitin-resistant (AMA-1) line. AMA-1 cells contain an α-amanitin-resistant DNA-dependent RNA polymerase II (refs 7–9). Cells were grown as monolayers in small plastic flasks in Dulbecco's MEM medium containing 10% foetal calf serum. For virus infection, cell sheets were washed with PBS, and allantoic fluid containing FPV was added to provide a multiplicity of infection of 10 PFU per cell. After 30 min adsorption, fresh MEM medium containing 2% foetal calf serum was added to the cells. Viral growth occurred in both cell types, and newly synthesised virus was detected either by haemagglutinin (HA) or plaque assay; maximum yields were obtained by 12 h after infection. The effects of increasing doses of α-amanitin in the culture medium on the 24-h yields of FPV from wild-type and AMA-1 cells, as determined using the HA assay, are shown in Fig. 1. Similar results were obtained when yields were measured by plaque assay. A dose-dependent inhibition of virus yield was observed only in wild-type cells. α-Amanitin (20 μg ml^{-1}) in the culture medium produced >95% reduction in the yield of influenza virus from wild-type CHO cells, but had no effect on the yield of virus from AMA-1 cells. These results indicate that the growth and release of influenza virus in AMA-1 cells is unsusceptible to α-amanitin.

To confirm that the DNA-dependent RNA polymerase II of AMA-1 cells is resistant to α-amanitin, in both uninfected and infected cells, the effect of α-amanitin on enzyme activity in isolated cell nuclei was determined. The polymerase II activity of nuclei extracted from uninfected wild-type and AMA-1 cells, both in the presence and absence of α-amanitin (5 μg ml^{-1}) was determined using the method of Lobban and Siminovitch[8]. A similar experiment was carried out using nuclei from both cell types infected 1 h previously with FPV, and the results are shown in Table 1. α-Amanitin reduced polymerase activity of both uninfected and infected wild-type CHO cells by 70–75%. The drug had no effect on polymerase levels of uninfected and infected AMA-1 cells. Virus infection seemed to increase enzyme activity by 20–30% at 1 h after infection.

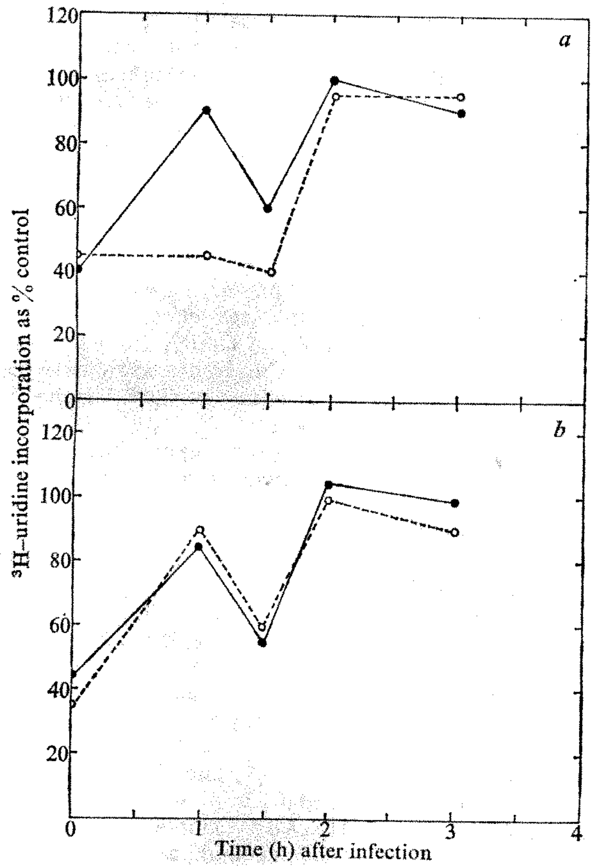

Fig. 2 Effect of α-amanitin on RNA synthesis in FPV-infected CHO cells. Confluent monolayers of wild-type and AMA-1 CHO cells were prepared in small, plastic flasks and infected with FPV at an input multiplicity of 10 PFU per cell. At various times, duplicate infected and control cultures were treated with α-amanitin 20 μg ml^{-1} for 1 h, followed by a 20-min pulse of ^{3}H-uridine (5 μCi ml^{-1}, 27.6 Ci mmol^{-1}). Cells were then washed with TCA and the amount of insoluble radioactivity determined by scintillation counting. The results are expressed as percentage counts incorporated into

$$\frac{\text{α-amanitin-treated FPV-infected CHO cells}}{\text{α-amanitin treated, uninfected cells}} \quad (\bigcirc)$$

and are compared with the percentage counts incorporated into

$$\frac{\text{FPV-infected CHO cells}}{\text{Uninfected cells}} \quad (\bullet)$$

a, Wild-type cells; *b*, AMA-1 cells.

In an attempt to establish the stage of the virus replication cycle affected by α-amanitin, virus-induced RNA synthesis was determined in infected wild-type and AMA-1 cells in the presence and absence of α-amanitin. Infected cells were exposed to an inhibitory dose of α-amanitin at various times following infection, and some time later their ability to synthesise RNA was determined. This experiment was carried out by adding α-amanitin (20 μg ml^{-1}) to the culture medium for 1 h before infection, or for hourly

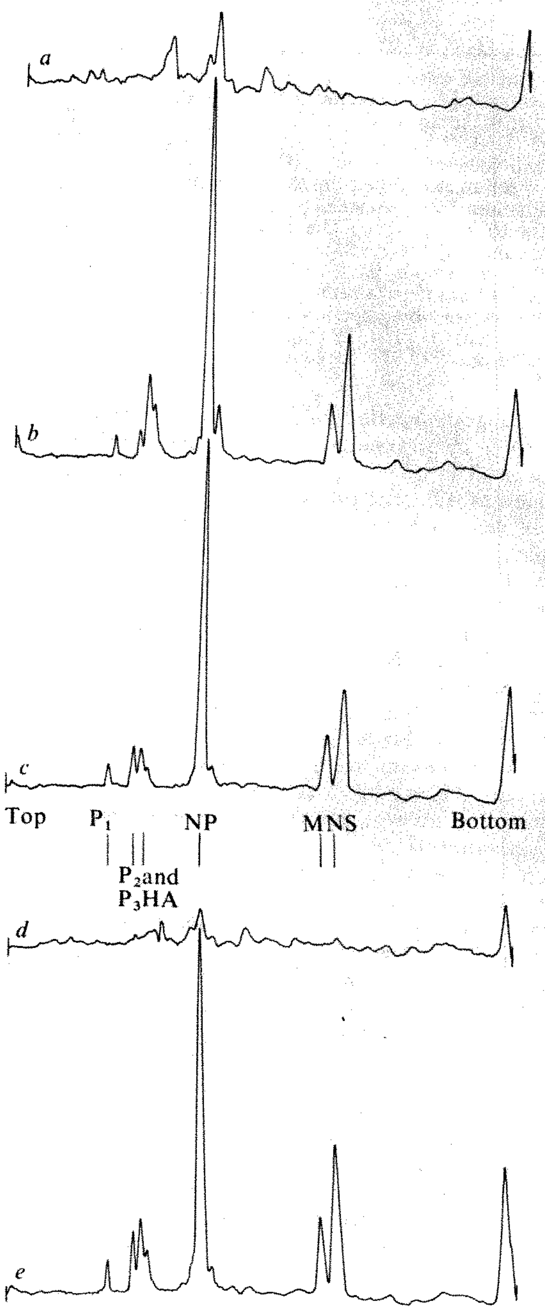

Fig. 3 Polyacrylamide slab gel electrophoresis of ^{35}S-methionine-labelled, high molecular weight polypeptides synthesised by uninfected and FPV-infected CHO cells. *a*, Uninfected wild-type CHO cells; *b*, wild-type CHO cells at 3¼ h after infection by FPV (10 PFU per cell); *c*, infected AMA-1 cells at 3¼ h; *d*, infected wild-type CHO cells at 3¼ h, α-amanitin 20 μg ml^{-1} present in the medium throughout infection; *e*, infected AMA-1 cells at 3¼ h, α-amanitin 20 μg ml^{-1} present in the medium throughout infection. Cells were infected or mock-infected and at 3 h received ^{35}S-methionine 5 μCi ml^{-1} for 15 min, before being processed for electrophoresis[12]. The material was loaded on to a 15% SDS–acrylamide gel, run for 16 h at 50V and processed for autoradiography. Densitometer tracings of the resulting autoradiographs are shown; the direction of migration and location of virus-induced polypeptides is indicated.

Table 1 Effect of α-amanitin on RNA polymerase II activity* of uninfected and FPV-infected CHO cells (1 h after infection)

Source of nuclei	α-Amanitin (μg ml^{-1})	% Control (c.p.m.)†
Wild-type uninfected	0	100
	5	37
Wild-type infected	0	120
	5	30
AMA-1 uninfected	0	100
	5	103
AMA-1 infected	0	131
	5	127

*Polymerase reaction mixture is based on that described by Lobban and Siminovitch[8]. In a volume of 560 μl were mixed 20 μmol Tris-HCl (*p*H 7.9); 0.7 μmol $MnCl_2$, 37.5 nmol each of ATP, CTP and GTP, 10 μCi ^{3}H-UTP at 40 Ci mmol^{-1} specific activity; 1.1 μmol β-mercaptoethanol; 240 μmol $(NH_4)_2SO_4$, and about 150 μg of purified cell nuclei. When the assay was carried out in the presence of α-amanitin, samples were pre-incubated with 5 μg ml^{-1} of the drug for 10 min at 37 °C. The mixture was then incubated at 30 °C for 15 min, and the reaction was terminated by addition of 1 ml $Na_4P_2O_7$ containing 0.2% bovine serum albumin. Acid-soluble radioactivity was determined by the method of Mahy and Bromley[11].

†Controls for this experiment were wild-type uninfected nuclei (2.36×10^5 c.p.m. ^{3}H-UTP mg^{-1} nuclear protein) and AMA-1-uninfected nuclei (2.57×10^5 c.p.m. mg^{-1}).

intervals from the time of infection and then exposing the cells to a 20-min pulse of ^{3}H-uridine (5 μCi ml^{-1}, 27.6 Ci mmol^{-1}). The level of TCA-insoluble radioactivity thus obtained at different times following infection in the drug-treated cells, was compared with that obtained in infected, untreated cultures. The results are shown in Fig. 2. Two peaks of virus-induced RNA synthesis (early and late) could be detected in both cell lines in the absence of α-amanitin, as has been described previously in FPV-infected primary chick cells[6,10]. AMA-1 cells treated with α-amanitin are indistinguishable from untreated cells, but the presence of α-amanitin inhibits the early peak of virus-induced RNA synthesis in wild-type CHO cells. The results suggest that the α-amanitin-sensitive step, mediated by DNA-dependent polymerase II, occurs early in infection.

To determine whether functional virus mRNA is made in α-amanitin-treated cells, the synthesis of virus polypeptides in infected wild-type and AMA-1 cells was investigated. Both cell types were infected with FPV in the presence or absence of α-amanitin (20 μg ml^{-1}) and at 3 h after infection received ^{35}S-methionine (5 μCi ml^{-1}) for 15 min. The cells were then processed for polyacrylamide gel electrophoresis and autoradiography according to the method described by Inglis *et al.*[12]. Densitometer tracings of the resulting autoradiogram are shown in Fig. 3. α-Amanitin reduced protein synthesis in infected wild-type cells to a level below that seen in uninfected cells. Virus-induced protein synthesis in α-amanitin-treated AMA-1 cells was quantitatively and qualitatively indistinguishable from that in cells untreated with α-amanitin.

The results reported here indicate that transcription of cell DNA by DNA-dependent RNA polymerase II is essential for the replication of influenza viruses. This transcription is an early event, apparently preceding the synthesis of virus-specific mRNA.

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Dopamine selectively increases ^{3}H-GABA release from slices of rat substantia nigra *in vitro*

THE firing rate of the dopaminergic neurones in the substantia nigra (s. nigra) of mammalian brain is controlled largely by descending striatonigral neurones. But new evidence suggests that the activity of nigral neurones may also be modulated by local circuits within the s. nigra, involving a local dendritic release of dopamine[2]. The demonstration that dopamine is present in[3], and released[4-6] from, the dendrites of nigral neurones both *in vitro* and *in vivo* supports this concept. The precise site of action of dopamine in the s. nigra is not known, but a dopamine-sensitive adenylate cyclase has been described in this brain region[7], apparently located presynaptically on the terminals of striatonigral afferents[8-10] containing either γ-aminobutyric acid (GABA) or substance P[11,12]. To determine whether presynaptic dopamine receptors in s. nigra are associated with substance P or GABA-containing axon terminals, we have investigated the effects of dopamine on the release of substance P and GABA from superfused slices of rat s. nigra *in vitro*. Our results show that dopamine stimulates selectively the release of GABA from nerve terminals in s. nigra.

Substantia nigra tissue (weighing approximately 6 mg, from one brain) was dissected from 0.8 mm coronal sections of fresh chilled rat brain. Initially the high affinity uptake of ^{3}H-GABA into 0.2×0.2-mm slices of s. nigra was measured, using the method of Iversen and Johnston[13]. After 10 min of incubation with ^{3}H-GABA (10^{-7} M) at 37 °C, a tissue : medium ratio of 72.9 ± 2.1 (mean ± s.e.m.; $n = 4$) was found, indicating that slices of s. nigra accumulated exogenous GABA avidly from the external medium.

Substance P release from superfused nigral slices was measured as described previously[14]. In experiments to measure GABA release, nigral tissue from two rats was pooled, incubated with ^{3}H-GABA, and the release of ^{3}H-GABA was measured in serial 2 min superfusate fractions (Fig. 1). The efflux of ^{3}H-GABA exhibited a rapid initial decline and after 15 min stabilised at a level which represented a loss of 0.7–0.9% of total tissue stores of ^{3}H-GABA per minute. Addition of depolarising concentrations of potassium chloride (11–47 mM) to the superfusing medium for 4 min produced a concentration-dependent release of ^{3}H-GABA. The potassium-evoked release of H-GABA was abolished in media with reduced Ca^{2+} and elevated Mg^{2+} concentrations (Table 1*a*). Okada and Hassler[15] described an electrically-evoked release of GABA from slices of rat s. nigra *in vitro*, but failed to demonstrate the calcium-dependence of this phenomenon.

Addition of dopamine (5–500 μM) to the superfusing medium for 4 min also produced a concentration-dependent increase in the rate of ^{3}H–GABA release. (Fig. 1*a*, Table 1*c*) and this effect was completely abolished in media with reduced Ca^{2+} and elevated Mg^{2+} concentrations. The actions of dopamine in both the corpus striatum and s. nigra

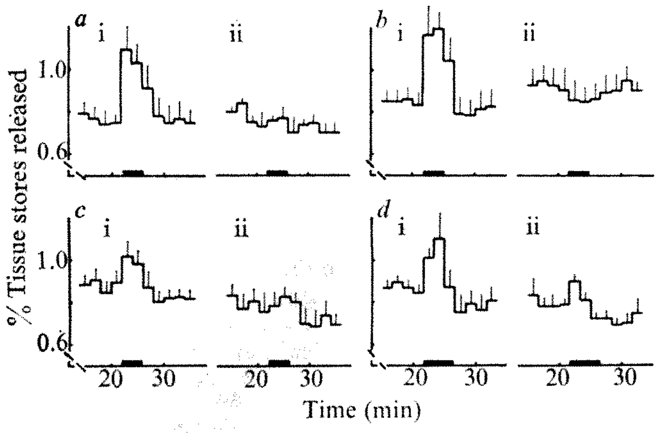

Fig. 1 Release of ^{3}H-GABA from rat substantia nigra. Slices of rat substantia nigra (0.2 × 0.2 mm) were incubated for 20 min in Krebs bicarbonate solution containing 10^{-7} M 3H-GABA (Radiochemical Centre, Amersham; specific activity 12.6 Ci mmol^{-1}). After incubation they were transferred to a superfusion apparatus[4] and washed at 37 °C with Krebs bicarbonate (containing 30 mg l^{-1} ascorbic acid and 10 mg l^{-1} EDTA to inhibit the spontaneous breakdown of added dopamine), at a rate of 0.5 ml per min. Superfusate samples were collected at 2 min intervals and the tritium content was determined. Nigral tissue was recovered at the end of the superfusion and the total content of radioactivity measured. ^{3}H-GABA efflux is expressed as a fractional rate constant, which represents the amount of ^{3}H released per minute in each sample as a percentage of the total tissue content of ^{3}H-GABA. In similar experiments over 90% of the radioactivity recovered in tissue and superfusate fractions was found to represent unchanged ^{3}H-GABA[21]. *a*, i, Release of ^{3}H-GABA evoked by addition of 50 μM dopamine (4 min, black bar, $n = 8$). ii, Effect of 50 μm dopamine in medium containing 0.1 mM Ca^{2+} and 12 mM Mg^{2+} ($n = 4$). *b*, Effect of α-flupenthixol (1 μM) on dopamine-evoked ^{3}H-GABA release. i, 50 μM dopamine pulse (4 min, black bar, $n = 5$). ii, Dopamine 50 μM (4 min) after addition of α-flupenthixol (1 μM) from min 18 to 24 ($n = 5$). *c*, Dexamphetamine-evoked release of ^{3}H-GABA. i, Amphetamine 50 μM (4 min, black bar, $n = 6$). ii, Amphetamine 50 μM after addition of α-flupenthixol (1 μM) for the total period of superfusion ($n = 4$). *d*, Evoked release of ^{3}H-GABA by addition of dibutyryl-cyclic AMP (db cAMP). i, db cAMP (1.2 mM) for 6 min (black bar), $n = 4$. ii, db cAMP (1.2 mM) in medium containing 0.1 mM Ca^{2+} and 12 mM Mg^{2+} ($n = 4$).

are mediated by dopamine receptors linked to a specific dopamine-sensitive adenylate cyclase that is potently antagonised by neuroleptic drugs[7,16,17]. Addition of the neuroleptics α-flupenthixol (1 μM) (Fig. 1*b*) or fluphenazine (1 μM) (Table 1*d*) to the superfusing medium completely abolished the dopamine-evoked release of ^{3}H-GABA. Haloperidol (1 μM) (Table 1*e*) also markedly suppressed the dopamine-evoked release. The potent dopamine agonist 4 - amino,6 - 7 - dihydroxy - 1,2,3,4 - tetrahydronaphthalene (ADTN) (ref. 18) (50 μM) mimicked the stimulatory effect of dopamine on ^{3}H-GABA release (Table 1*f*). The dopamine-evoked release of ^{3}H-GABA in the s. nigra thus seems to be mediated by an action on dopaminergic receptors with pharmacological specificity similar to that of receptors in other dopamine-rich brain areas.

Addition of dexamphetamine (50 μM) also caused a small but significant ($P<0.05$) release of ^{3}H-GABA from slices of s. nigra (Fig. 1*c*), and this effect could be blocked by previous exposure to α-flupenthixol (1μM). Amphetamine lacks any direct action on central nervous system (CNS) dopamine receptors[18], but stimulates a release of tissue stores of dopamine in various regions of CNS including s. nigra[2,19]. The stimulatory effect of amphetamine suggests that a release of endogenous dopamine from the dendrites of nigral neurones can stimulate a local release of GABA. A functional dendro–axonic synaptic interaction may thus exist even in superfused slices of s. nigra maintained *in vitro*.

Superfusion with the stable cyclic AMP analogue, dibutyryl-cyclic AMP (1.2 mM) also caused a calcium-dependent increase in ^{3}H-GABA release (Fig. 1*d*), showing that the effect of dopamine may be related to increases in intracellular cyclic AMP levels in GABA-containing nerve terminals. Minneman and Cuello (personal communication) have observed presynaptic localisation of cyclic nucleotide phosphodiesterase in the rat s. nigra, providing further evidence for the localisation of a cyclic AMP system within nerve terminals in the s. nigra.

Although there is evidence for an involvement of cyclic AMP, the cellular mechanism responsible for the dopamine-evoked release of GABA remains unknown. The calcium dependence of this phenomenon suggests a possible similarity to the mechanism involved in the normal stimulus-evoked release of transmitters. Furthermore, when nigral slices were exposed simultaneously to dopamine (50 μM) and to high potassium (11 mM) the effects on ^{3}H-GABA release were not additive (Table 1*b*). In parallel experiments the spontaneous efflux of substance P was found to be 28.36±4.08 fmol mg^{-1} (mean±s.e.m; $n=4$); addition of dopamine (50 μM) for 4 min produced no significant change in spontaneous efflux (29.84±0.88 fmol mg^{-1}, $n=4$). Similarly the potassium-evoked release of substance P (36.41±4.07 fmol mg^{-1}; $n = 3$) was unaffected by simultaneous addition of dopamine (39.26±6.13 fmol mg^{-1}; $n = 3$).

Since high affinity GABA uptake occurs into glial cells as well as neurones, it is possible that the GABA release we have described occurred partly from glial cells. But, the apparent localisation of dopamine-sensitive adenylate cyclase on striatonigral nerve terminals suggests that dopamine released within the s. nigra produces a selective stimulation of the release of GABA located within inhibitory striatonigral terminals, and this might provide the basis for a local inhibitory feedback circuit. The inhibitory effects of dopamine when applied iontophoretically into the s. nigra[20] may, at least in part, be mediated by such an effect of dopamine on GABA-containing axon terminals. The lack of effect of dopamine on substance P release suggests that the dopamine-sensitive adenylate cyclase in rat s. nigra may be localised predominantly on GABA-containing axon terminals.

ADTN was provided by Dr T. Inch, Chemical Defence Research Establishment, Porton Down, and substance P by Dr R. Hirschmann, Merck, Sharpe and Dohme. T.M.J.

Table 1 Effect of dopamine (DA) and high potassium (K^+) on release of ^{3}H-GABA from rat substantia nigra

Stimulus	Evoked release of ^{3}H-GABA (% of total tissue stores)
a K^+ 11 mM	3.78±0.56 (4)
K^+ 23 mM	5.94±1.02 (4)
K^+ 47 mM	9.70±1.88 (4)
K^+ 11 mM; Ca^{2+} 0.1 mM, Mg^{2+} 12 mM	0.08±0.12 (4)
b K^+ 11 mM + DA 50 μM	3.40±0.62 (4)
c DA 5 μM	1.46±0.38 (5)
DA 50 μM	2.24±0.40 (8)
DA 500 μM	3.00±1.16 (4)
d DA 50 μM + fluphenazine 1 μM	0.06±0.08 (4)
e DA 50 μM + haloperidol 1 μM	0.42±0.18 (4)
f ADTN 50 μM	1.52±0.42 (4)

Values are means ± s.e.m. calculated as the total evoked release of ^{3}H-GABA in the 8-min period during and after application of a 4-min stimulus. The evoked release was calculated by subtraction of the average value for spontaneous ^{3}H-GABA efflux during the 6-min immediately before application of the stimulus (0.7–0.9% per min).

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Müller cell localisation of glutamine synthetase in rat retina

IN retina as in brain, the metabolism of glutamic acid and γ-aminobutyric acid (GABA) is compartmentalised in biochemically defined pools[1–4]. When retinas are incubated *in vitro* with radioactive glutamate and GABA, the specific activity of the glutamine produced is greater than the specific activity of the total tissue glutamate. This indicates that glutamine is formed from a small glutamate pool of higher specific activity than the total glutamate pool. To understand better the metabolic organisation of these amino acids in retina, the anatomic localisation of the small glutamate pool is essential. There is evidence that Müller cells (retinal glia) may be the site of the small glutamate pool. Autoradiographic studies indicate that mammalian Müller cells take up GABA and glutamate[5–8]. Moreover, these cells contain succinic semialdehyde dehydrogenase[9] and GABA-transaminase[10] which are enzymes involved in the degradative metabolism of GABA. In a number of species, glutamine synthetase (GS), a key enzyme of the small glutamate pool[11], shows a striking increase in activity[12–14] which parallels the morphological development of the Müller cell[15–17]. We describe here the localisation of GS to Müller cells in rat retina by immunohistochemical techniques.

Normal adult albino rats were lightly anaesthetised with ether, and 1 ml of 1% sodium nitrite and 1,000 units of heparin sodium were injected intravenously. One minute later, rats were perfused for 15 min through the ascending aorta with 4% paraformaldehyde in 0.1 M sodium cacodylate buffer, *p*H 7.4, at room temperature. After perfusion, eyes were enucleated, bisected along the sagittal plane, and the vitreous body removed. Retinas were washed overnight at 4 °C in phosphate buffered saline (PBS) containing 10% sucrose. Retinas were then rinsed for 15 min in PBS containing 12% glycerol and 4% sucrose before quick freezing in a dry ice–95% ethyl alcohol mixture. Sections (10 μm thick) were obtained, treated with antibody to GS and processed with the indirect peroxidase-labelled antibody method[18,19]. Details of the preparation of the anti-GS antibody, its purification and characterisation have been described previously[20]. Briefly, immunoelectrophoretically proved monospecific antibody to sheep GS was prepared in rabbits and purified by affinity chromatography[21]. Rat and sheep GS were antigenically identical as determined by double immunodiffusion. Control sections were treated the same as experimental sections except that affinity-purified antibody to the soluble component of basement membrane (kindly provided by Dr Antonio Martinez-Hernandez) was substituted for the anti-GS antibody.

Sections of retina treated with anti-GS antibody and processed with the indirect peroxidase-labelled antibody

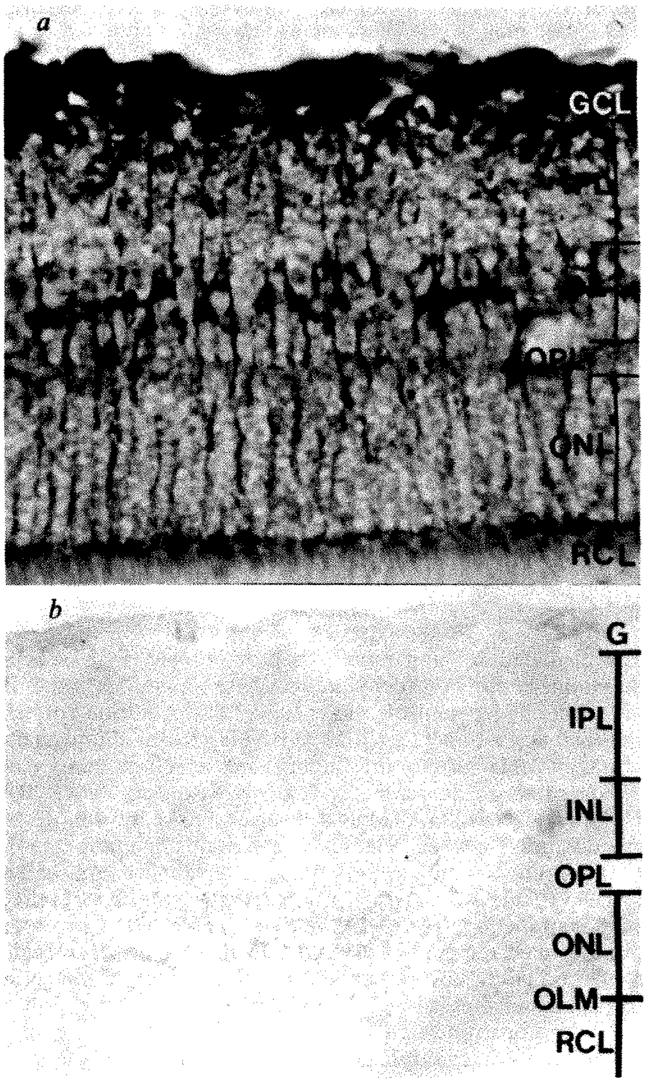

Fig. 1 *a*, Photomicrograph of rat retina cryostat section stained for glutamine synthetase by the indirect peroxidase-labelled antibody method. In the inner nuclear layer (INL), reaction product is conspicuous in Müller cell perikarya. Positive Müller cell processes extend in opposite directions from the inner nuclear layer. Densely stained areas are present in the ganglion cell layer (GCL) while small beads of reaction product form a thin line along the outer limiting membrane (OLM). RCL, rod and cone layer; ONL, outer nuclear layer; OPL, outer plexiform layer; IPL, inner plexiform layer. (× 300.) *b*, Control section: rat retina treated similarly to section in Fig. 1*a* except antibody to basement membrane was substituted for GS antibody. No reaction product is present. (× 300.)

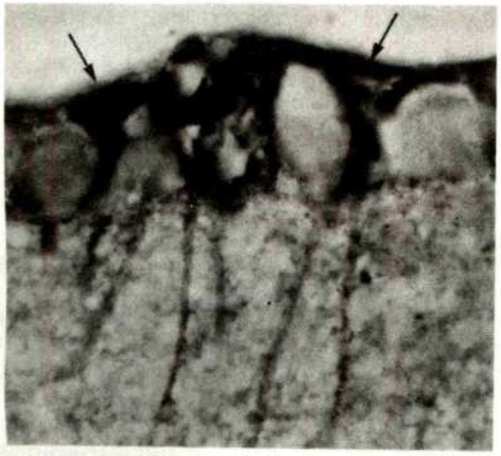

Fig. 2 Higher magnification of the ganglion cell layer demonstrates characteristic funnel-shaped Müller cell processes (arrows) containing deposits of reaction product. There is no staining in ganglion cells. (×750.)

method demonstrated that GS was confined to Müller cells (Fig. 1*a*). Peroxidase reaction product in the perikaryon clearly outlined the polygonal Müller cell nuclei located in the inner nuclear layer. Positive staining Müller cell processes were traced from the inner nuclear layer through the inner plexiform layer as narrow parallel columns ending in funnel-shaped deposits in the ganglion cell and nerve fibre layers (Fig. 2). Positive Müller cell processes also extended through the outer plexiform and outer nuclear layers to terminate as distinct beads of reaction product along the outer limiting membrane. The presence of GS in other glial elements could not be excluded. No reaction product was seen in photoreceptors, horizontal cells, bipolar cells, amacrine cells, or ganglion cells. Staining was not observed in retinas treated with control antibody (Fig. 1*b*).

This study has shown that retinal GS in the rat and therefore the small glutamate compartment associated with glutamine synthesis is localised to Müller cells, supporting the suggestion of Kennedy and co-workers[2]. Analogous to this finding is the localisation of GS to glia in rat brain[20]. Perhaps an integral role can now be assigned to the Müller cell in the GABA–glutamate–glutamine cycle proposed to occur in retina[1]. According to this concept, Müller cells take up neuronally-released glutamate and GABA, thereby inactivating and conserving these amino acids which are subsequently converted to glutamine by the action of GS. Glutamine, which is freely diffusible, then leaves the Müller cell and is available to neurones for the formation of glutamate and GABA. The localisation of GS to Müller cells thus lends firm support for a crucial role of these cells in GABA and glutamate metabolism.

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Electrical stimulation of denervated muscles reduces incorporation of methionine into the ACh receptor

ONCE a neuromuscular synapse has formed, the density of acetylcholine (ACh) receptors in the subsynaptic muscle membrane remains relatively constant, even after denervation of the muscle[1]. In contrast, the density of ACh receptors in the extrajunctional muscle membrane can vary widely. Extrajunctional receptors are present at high density in developing muscle, but are reduced to low levels during maturation of muscle fibres[2]. Their number is increased after denervation in the adult[3,4], and is again decreased on subsequent re-innervation of the muscle[5]. Several experiments have demonstrated that the amount and pattern of muscle activity strongly influence the density of extrajunctional ACh receptors (for review, see ref. 6), although other factors may be important as well[6–9]. Because extrajunctional receptors undergo continual turnover in the membrane[10–12], the effect of changes in activity on their density could result from alteration in rates of receptor synthesis or degradation. Recent experiments have shown that the reduction of receptor levels caused by stimulation in organ culture is accompanied by a decrease in the rate of receptor degradation[13]. These results imply that activity decreases the rate of receptor synthesis or its insertion into the membrane. In direct support of this idea, we report that electrical stimulation of denervated muscle reduces incorporation of ^{35}S-methionine into ACh receptors.

Left hemidiaphragms from male Sprague–Dawley rats (50–60 g) anaesthetised with ether were denervated by intrathoracic transection of the phrenic nerve. Three or four days later, denervated hemidiaphragms with ribs attached were removed from the animals, pinned out in pairs in Sylgard lined culture dishes, and incubated in 12 ml Trowells T8 medium as described previously[10,13]. One hemidiaphragm of each pair received direct electrical stimulation by platinum electrodes recessed in the Sylgard beneath the muscle[13], whereas the other muscle was not stimulated. Suprathreshold square pulses of 2 ms duration and alternating polarity were applied to the muscles at 40 Hz for 350 ms once every 3 s. After 17–53 h in culture, the medium was changed and the muscles incubated for 1 h with Trowells medium modified to contain a low concentration of methionine (2×10^{-6} M). ^{35}S-methionine (0.5.0 mCi; 300–500 mCi mmol^{-1}; New England Nuclear) in modified T8 medium was then added to each dish and the muscles labelled for 6–12 h.

The incorporation of ^{35}S-methionine into the ACh receptor was determined after purification by affinity chromatography on cobrotoxin–Sepharose and concanavalin A (con A–Sepharose) as depicted in Fig. 1. Diaphragms were removed, blotted dry, weighed and homogenised. A

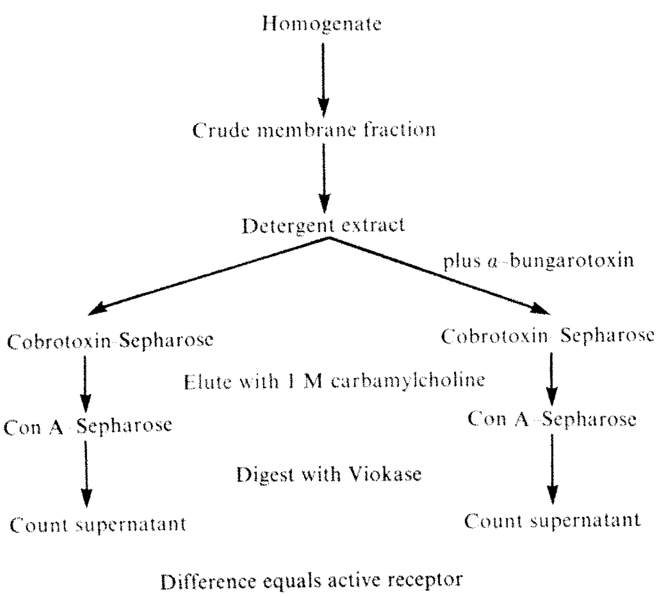

Fig. 1 Schematic outline of the procedure used to measure ^{35}S-ACh receptor. Details are provided in the text and in Table 1.

crude membrane fraction was extracted with Triton X-100 (ref. 14), and the extract was divided into two equal portions. Excess unlabelled α-bungarotoxin was added to one portion (final concentration 10^{-5} M), and both were incubated at room temperature for 15 min. The extracts were applied to parallel columns of cobrotoxin–Sepharose and eluted with carbamylcholine[14]. Incubation with unlabelled toxin before the column step specifically prevents adsorption of the receptor (Froehner & Hall, unpublished) and the eluate of the column receiving this extract contained only contaminating radioactivity, thus serving as a control for the paired eluate. Because the amount of contaminating radioactivity at this stage was large and variable, further purification was necessary. Since ACh receptor is a glycoprotein which binds to con A (ref. 15) additional purification was achieved by adsorbing each of the paired eluates with con A–Sepharose. Material retained on this column was digested with Viokase and the released radioactivity measured by liquid scintillation spectrometry.

Results from a typical experiment are shown in Table 1. The amount of radioactive receptor was calculated as the difference between each sample and its paired control. Ratios of radioactive receptor, corrected for wet weight, were then computed for each pair of stimulated and unstimulated muscles. Control experiments established that receptor from both stimulated and unstimulated muscles was recovered equally well from the cobrotoxin–Sepharose and was quantitatively retained on con A–Sepharose.

Results of ten experiments in which muscles were stimulated for 1–3 d are shown in Table 2. Stimulation of the muscles reduced the total amount of ACh receptor present in muscle extracts and reduced incorporation of ^{35}S-methionine into the receptor. Both the reduction in total amount of receptor and in the extent of incorporation were greater with longer periods of stimulation. Complete inhibition of synthesis was not observed even after 3 d. The residual synthesis probably reflects inadequate stimulation of some fibres[13], but could be due to suboptimal parameters of stimulation or to the presence or absence of some factor unrelated to activity.

Because the phasic pattern of stimulation used in these experiments was different from the stimulation (2–10 Hz) used in earlier experiments[13], we repeated our previous comparison of receptor degradation in stimulated and unstimulated muscles. When muscles were stimulated phasically, the rate of receptor degradation ($t_{1/2} = 12.08 \pm 0.8$ h, $n = 3$) was not significantly different from that found without stimulation ($t_{1/2} = 11.8 \pm 0.9$ h, $n = 3$). These results are in agreement with our previous conclusions that the decrease in ACh receptor levels in stimulated muscle is not caused by an increased rate of breakdown.

Incorporation of ^{35}S-methionine into richloroacetic acid (TCA)-precipitable material in the Triton X-100 extracts from stimulated muscles was identical to that found in control muscles (105±9%, mean±s.e.m., $n = 10$). Thus it is unlikely that differences in specific activity of intracellular methionine could account for the difference we have observed. Autoradiographs of two-dimensional gels[16] of the labelled extracts from stimulated and unstimulated muscles were almost identical, differing only by a few proteins. Since neither the major patterns of protein synthesis nor the total amount of isotope incorporated is changed by stimulation, the effect on ^{35}S-methionine incorporation into receptor must be relatively specific.

The amount and pattern of muscle activity is known to affect several other properties of muscle fibres besides extrajunctional ACh receptor level. These include the species of myosin light chains made by the fibres[17], the rate of synthesis of myosin heavy chain[18], the amount of acetylcholinesterase activity in the muscle[19] and several physiological properties of the muscle, the biochemical basis of which is not known[6]. Many of these changes seem to be controlled at the level of gene expression[20,21]. The mechanism by which muscle activity mediates these effects is a major question for future investigation.

Previous experiments have shown that incorporation of

Table 1 Determination of ^{35}S-receptor

	Stimulated c.p.m. per mg wet weight		Unstimulated c.p.m. per mg wet weight	
Homogenate (TCA-precipitable)	4.4×10^5		2.8×10^5	
Triton X-100 extract (TCA-precipitable)	1.5×10^5		1.1×10^5	
	−α-BUTX	+α-BUTX	−α-BUTX	+α-BUTX
Con A–Sepharose retained	8.2	6.2	28.6	11.4
Difference	2.0		17.2	

α-BUTX, α-Bungarotoxin.

Two left hemidiaphragms that had been denervated 4 d earlier were cultured for 3 d and labelled with 1 mCi ^{35}S-methionine for the final 12 h. The stimulated and unstimulated muscles weighed 66 and 78 mg, respectively. Each was homogenised in 1 ml 50 mM Tris-HCl, *p*H 7.4, 50 mM NaCl with 1 mM EDTA, 1 mM EGTA, 0.4 mM phenylmethylsulphonyl fluoride (PMSF), 20 U ml^{-1} Trasylol (Sigma) and 0.1 μg ml^{-1} Pepstatin. Homogenates were diluted to 5 ml with buffer and centrifuged at 48,000 r.p.m. for 45 min in a Spinco SW 50.1 rotor. The pellet was resuspended in 500 μl of the above buffer, Triton X-100 was added to a final concentration of 3% and extraction carried out on ice for 1 h. The extract was centrifuged at 20,000*g* for 1 h and the resultant supernatants were each divided into two equal portions. To one aliquot of extract from each muscle was added α-bungarotoxin (final concentration 10^{-5} M α-BUTX); followed by incubation of each portion for 15 min at room temperature. Four 0.3-ml cobrotoxin–Sepharose[14] (400 μg cobrotoxin per ml bed volume) columns were poured and washed consecutively with 50 mM Tris-HCl, *p*H 7.4, 1% Triton X-100 (TT) containing 1 M NaCl and with 50 mM Tris-HCl, *p*H 7.4, 50 mM NaCl and 1% Triton X-100 (TNT). The pre-incubated extracts were then adsorbed on to the columns at 4 °C, and the columns washed sequentially with TNT (5 ml), TT+1 M NaCl (10 ml) and TNT (5 ml). In each case no radioactivity was detectable in the final wash. ACh receptor was then eluted with several column volumes of TNT which contained 1 M carbamylcholine chloride, *p*H 8.6, and 0.4 mM PMSF, 20 U ml^{-1} Trasylol and 0.1 μg ml^{-1} Pepstatin. Duplicate 25-μl aliquots from 1-ml eluates were counted and the remainder adsorbed to pre-washed 0.5 ml con A–Sepharose columns (2 mg con A ml^{-1} bed volume). After the columns were washed with TNT to remove non-adsorbed radioactivity, the resin was mixed with 0.5 ml 3 mg ml^{-1} Viokase in TNT and incubated overnight at 37 °C. The con A–Sepharose was removed from suspension by centrifugation and the supernatant radioactivity determined.

Table 2 Effect of stimulation on ACh receptor synthesis

Duration of culture (h)	Duration of labelling (h)	Total ACh receptor stimulated/ unstimulated	^{35}S-ACh receptor stimulated/ unstimulated	n
24*	6	$81 \pm 4\%$	$42 \pm 7\%$	4
51†	6	$39 \pm 10\%$‡	$23 \pm 7\%$	3
66†	12	$28 \pm 8\%$‡	$14 \pm 2\%$§	3

*Denervated 3 d before culture.
†Denervated 4 d before culture.
‡Significantly different from 24 h stimulation $P < 0.005$.
§Significantly different from 24 h stimulation $P < 0.05$.

Effect of stimulation on ACh receptor synthesis. Culture duration is indicated. ^{35}S-methionine was present in the culture for the time shown just before termination of the experiment. Total ACh receptor was determined by a DEAE filter-binding assay of ^{125}I-α-bungarotoxin as described previously[14]. ^{35}S-ACh receptor determined as indicated in Table 1. Values expressed as the mean ±s.e.m. n equals the number of muscle pairs. In two cases (24 h and 66 h of culture, respectively) no difference (100%; 102%) in incorporation was observed between stimulated and control muscles. Total ACh receptor was reduced in both cases; therefore, either stimulation was effective only during part of the culture period before labelling, or recovery of radioactivity was not the same for control and stimulated muscles. Because of the large, statistically significant variation of these values from the others, results from these two experiments have been excluded from the data in Table 2. Their inclusion, however, would not affect the general conclusions to be drawn.

^{35}S-methionine into the ACh receptor can be detected in denervated muscles only after accumulation of extrajunctional receptor begins[22]. These results along with those reported here show that changes in incorporation of ^{35}S-methionine into ACh receptor parallel changes in overall receptor density and suggest that extrajunctional receptor levels are controlled by regulation either of synthesis or of some other step before appearance of the receptor in the external membrane.

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High conductance in an epithelial membrane not due to extracellular shunting

THERE is conclusive evidence for a cellular and extracellular route for ions across epithelial tissues[1]. The extracellular pathway is constituted by the tight junctions and the lateral intercellular space. Epithelia have been classified as 'tight' or 'leaky' depending on the ratio of the conductances of both pathways (that is, in a tight epithelium the ratio: $g_{\mathrm{shunt}}/g_{\mathrm{cell}} \leqslant 1$) Moreover, tight epithelia have transmural resistances and electrical potentials far greater than those observed in leaky epithelia. We report here that the epithelium of rabbit salivary duct does not confine to this rule since it combines several characteristics of tight epithelia with resistance values as low as in proximal tubules of the kidney, an extremely leaky epithelium. Permeability studies with polar non-electrolytes support the conclusion that the high conductance is not due to extracellular shunting. Our study implies that the magnitude of the epithelial resistance does not determine whether an epithelium is tight or leaky.

Main ducts of rabbit salivary glands were dissected and mounted in a perfusion chamber as before[2]. Transductal resistance was measured by a direct method. We developed a combined current passing and potential sensing electrode small enough to fit into the lumen of the duct. Details of the electrode are published elsewhere[3]. Ductal permeability to non-electrolytes was determined by conventional isotope techniques. Unidirectional fluxes of labelled compounds were measured from serosa to mucosa. Ringer solutions contained (in mM): Na, 145; K, 4; Mg, 1; HCO_3, 25; acetate, 10; pyruvate, 10; pH, 7.4. The other anions were Cl, 100 or SO_4, 50 respectively. Sulphate solutions have mannitol to restore isotonicity and also 2 mM Cl for proper functioning of the Ag/AgCl electrode[3]. The serosal solution contained in addition 6 mM glucose and protein (Haemacel, 30 g l^{-1}) to keep ductal transport optimal[2].

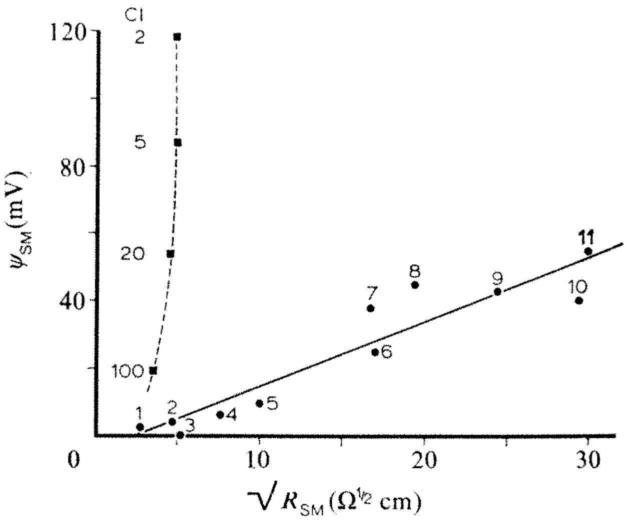

Fig. 1 Relationship between transepithelial potential (ψ_{SM}) and the square root of resistance in various epithelia (straight line). (1), Proximal tubule, rabbit (ref. 4); (2), ileum, rabbit (ref. 5); (3), gall bladder, rabbit (ref. 6); (4), gall bladder, human (ref. 7); (5), early distal tubule, rat (ref. 8); (6), colon, rabbit (ref. 9); (7), gastric mucosa, lizard (ref. 10); (8), late distal tubule, rat (ref. 11); (9), late distal tubule, dog (ref. 12); (10), collecting duct, rabbit (ref. 13); (11), collecting duct, hamster (ref. 14). The dashed line gives values of ψ_{SM} and $\sqrt{R_{SM}}$ measured for rabbit main duct in solutions with varying Cl-concentrations. Solutions were symmetrical and the number indicate Cl concentration. In low-Cl solutions current pulses induced strong polarisation effects and voltage responses were extrapolated to $\mathrm{d}V/\mathrm{d}I$ ($t \to 0$). In high-Cl solutions polarisation effects were minimal. Our resistance values in high Cl solutions agree well with values obtained from applying cable theory as reported by Fromter *et al.*[24]. $y = 1.89x - 4.2$; $r = 0.94$.

In Fig. 1 electrical potentials (Ψ_{SM}) are plotted against the square root of resistance values ($\sqrt{R_{SM}}$) reported for various epithelial tissues. The correlation between these parameters is highly significant, indicating that the smaller the extracellular shunt conductance the higher the values for Ψ_{SM} and R_{SM}. The dashed line in Fig. 1 gives that same relation for salivary duct epithelium, which shows a totally different behaviour. Besides the fact that Cl ions have a strong short circuiting effect on the potential we observed in SO_4 solutions potentials up to 140 mV combined with resistance values as low as 10 Ω cm^2. Potentials of the same magnitude have been reported only for tight epithelia with resistance values three orders of magnitude greater[16]. Cl ions have also a short-circuiting effect on frog skin potentials, although less pronounced[15].

In Fig. 2 values of Ψ_{SM} and $\sqrt{R_{SM}}$ are plotted as a variation among 23 main ducts, measured immediately after mounting in SO_4 solutions. There is a statistically significant inverse relationship between these two parameters. A similar inverse relationship has been reported for urinary bladders, however, only when special measures were taken to exclude edge damage effects[16,17]. Evidently an inverse relationship will not occur when a 'passive' extracellular shunt determines the variation in the transepithelial resistance. The phenomenon can be explained when the rate of active Na-transport is regulated by the Na-conductance of the luminal cell membrane[16]. A greater Na conductance would then evoke higher rates of Na pumping in the contraluminal membrane. Figures 1 and 2 show clearly that the main duct combines tight epithelial properties with resistance values normally observed in very leaky epithelia. The results given in Table 1 support this ambiguity. Permeabilities to polar non-electrolytes in the main duct are of the same order as those in the toad urinary bladder. The apparent impermeability to inulin suggests that a significant paracellular shunt for non-electrolytes is absent. On the other hand, a greater P_{Cl} value reflects the low resistance of the duct.

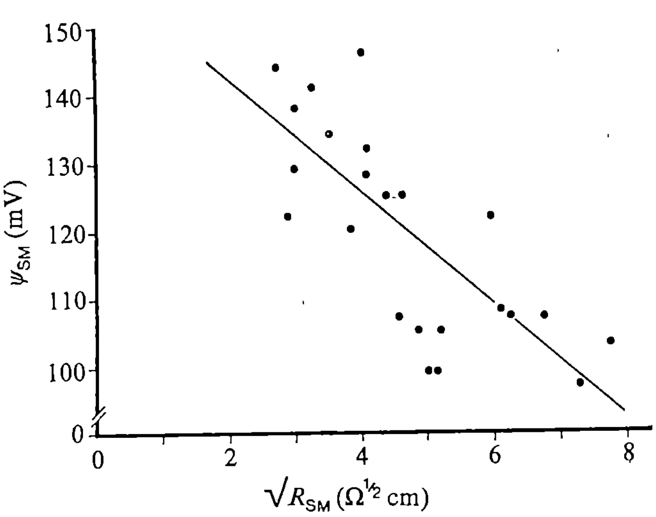

Fig. 2 Inverse relationship between potential difference (ψ_{SM}) and resistance ($\sqrt{R_{SM}}$) as a variation among 23 main ducts measured in symmetrical SO_4 solutions with 2 mM Cl. $y = -8.25x + 158.3$; $r = 0.75$.

Three more arguments are in favour of ranking ductal epithelium as tight despite the low resistance. The natriuretic drug amiloride inhibited net Na-transport in salivary duct completely when it was applied to the mucosal solution (10^{-4} M). In SO_4-solutions ductal resistance increased from 22.3±1.8 to 91.4±12.6 Ω cm^2 ($n = 7$) after addition of amiloride. In Cl-solutions the increase was less pronounced (from 6.6±0.4 to 14.1±0.7 Ω cm^2, $n = 4$). The relative increase in resistance is comparable with amiloride treatment of frog skin[16].

Table 1 Permeability of main duct, urinary bladder and gallbladder to polar non-electrolytes

	Rabbit submaxillary main duct	Toad urinary bladder	Rabbit gall bladder (ref. 19)
Solute	(10^{-7} cm s^{-1})	. (10^{-7} cm s^{-1})	(10^{-7} cm s^{-1})
Urea	56	14 (ref. 18)	890
Sucrose	6.5	5.1 (ref. 17)	76
Inulin	n d.	n.d. (ref. 17)	29
Cl^-	550 (ref. 21)	13 (ref. 17)	—

The tubular perfusion rate was 12.5 µl min^{-1}. P values of main duct are quoted as the mean estimated from five estimates (n.d., not detectable). P_{Cl} was determined in ducts with open circuit. P_{Cl} was independent of the electrical potential (for detailed discussion see ref. 21).

In Cl-solutions we measured a net Na-absorption rate of 587 nEq min^{-1} cm^{-2}, comparable with 683 nEq. min^{-1} cm^{-2} reported by Martin *et al.*[22], corresponding with 0.94–1.10 mA cm^{-2}. Potential and resistance values in this condition were 12.3±2.4 mV and 10.8±1.9 Ω cm^2 ($n = 9$). From these values a short-circuit current (SCC) of 1.14 mA cm^{-2} can be calculated which equals net Na-transport. The SCC is only equal to net Na-transport in tight epithelia[17,23]. Furthermore, salivary duct epithelium responds asymmetrical to cation gradients contrasting with symmetrical responses in leaky epithelia[24].

Interestingly, the resistance of cell membranes can be as low as 10 Ω cm^2—we found no reason to ascribe this low resistance to an extracellular shunt pathway.

From the unusual combination of low resistance and electrical properties of tight epithelia we have to conclude that the only criterion whether an epithelium is 'tight' or 'leaky' is the conductance ratio of the cellular and extracellular route since the electrical resistance alone is not sufficient to discriminate between the two classes of epithelia.

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Fluorescent probe detects redistribution of cell calcium during stimulus–secretion coupling

STIMULUS-secretion coupling in pancreatic acinar cells is believed to be mediated by a rise in intracellular Ca^{2+} activity[1–5]. Based on the observation that physiological secretagogues trigger a rapid increase of Ca^{2+} efflux[3,4] and stimulate enzyme release even in the absence of extracellular calcium[2,4,5], it has been proposed that stimulus–secretion coupling involves a redistribution of intracellular calcium[3,5,6]. Since most calcium in pancreatic acinar cells is bound to membranes or is sequestered in mitochondria[7], we have attempted to monitor these calcium stores during secretion by using the fluorescent probe chlorotetracycline (CTC). This probe forms highly fluorescent complexes with Ca^{2+} and Mg^{2+} in the presence of biological membranes or detergent miceles[8,9] and has been successfully used in isolated mitochondria[10,11] and sarcoplasmic reticulum vesicles[12] to monitor calcium movements induced by substrates and inhibitors. In this report, we show that fluorescence of CTC associated with pancreatic acinar cell membranes decreases rapidly on stimulation of enzyme secretion and that this intensity change is preferential for CTC complexed to calcium.

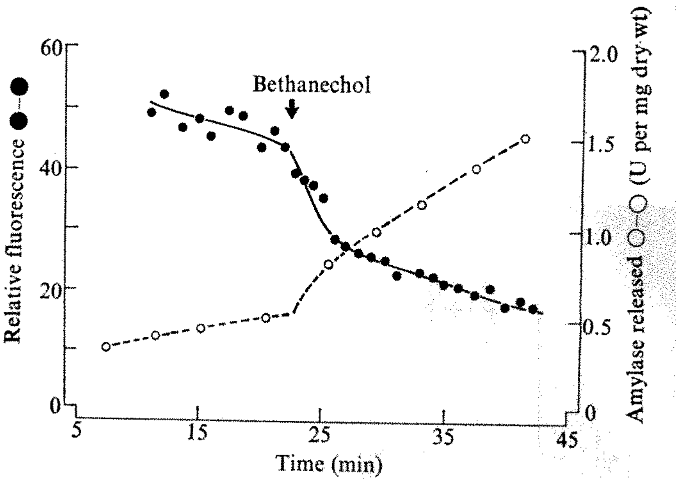

Fig. 2 CTC fluorescence of and amylase release from dissociated pancreatic acini. Acini were loaded with CTC as described in Fig. 1, then stirred periodically in a fluorometer cuvette maintained at 37 °C. Fluorescence was monitored as in Fig. 1. Amylase released into the medium was measured by removing 0.2 ml aliquots of the suspension at the designated times, centrifuging at 8000*g* for 20 s, and assaying amylase in the supernatant by the method of Rinderknecht *et al.*[16]. Amylase activity is expressed in international units per mg dry wt of acini; the cuvette contained 1.47 mg dry wt of acini ml^{-1}. Addition of bethanechol at the arrow (final concn = 1.25×10^{-4} M) elicited a rapid decrease in fluorescence and a 240% increase in the rate of amylase release. These results are representative of six similar experiments.

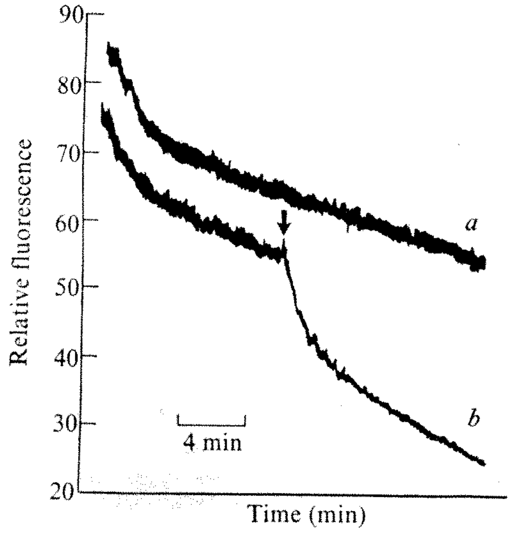

Fig. 1*a*, Effect of bethanechol on the fluorescence of dissociated acini loaded with chlorotetracycline (CTC). Enzymatically dissociated acini were prepared from the pancreata of 8 to 12 fasted male Swiss white mice by the method of Amsterdam and Jamieson[14] modified to exclude the Ca^{2+} chelation step[15]. Acini were preincubated for 60 min in a Tris-buffered Ringer (TR)[2] containing 1% bovine serum albumin (BSA) and 10^{-4} M CTC. Acini were then washed and resuspended in 4 ml of TR containing no BSA or CTC and the suspension placed in a cuvette in an Aminco-Bowman spectrofluorometer having a band pass of 15 nm and modified so as to allow continual stirring and temperature control at 37 °C. Excitation of CTC fluorescence was at 400 nm and emission monitored at 525 nm. *b*, Bethanechol (final concn = 10^{-4} M) was added at the arrow and resulted in a rapid loss of fluorescence intensity. This experiment was representative of 22 similar experiments.

Enzymatically-dissociated pancreatic acini were pre-incubated 60 min in 10^{-4} M CTC, then resuspended in buffer containing no CTC and placed in a fluorometer cuvette. The fluorescence intensity of these acini decreased with time, the decrease usually being faster in the first 5 min of resuspension and slower thereafter (Fig. 1*a*). Application of the cholinergic agonist, bethanechol, during the slower phase (Fig. 1*b*) elicited a marked loss of fluorescence occurring largely in the first 6 min of secretagogue action. After this time, fluorescence intensity continued to decrease at a rate similar to that before bethanechol addition. The secretagogue-induced fluorescence decrease and a stimulation of amylase release into the medium could be measured simultaneously on acini incubated in a cuvette as shown in Fig. 2. As observed previously, an increased loss of fluorescence occurred in the 5 min following bethanechol addition. At the same time, the rate of amylase release measured 5–15 min after the addition of bethanechol was increased by 240% over the control rate measured in the 15 min before adding bethanechol. This suggests that the CTC present in these acini did not interfere with normal stimulation of secretion, a result supported by experiments in which acini pre-incubated 60 min with and without 10^{-4} M CTC released identical amounts of amylase in response to bethanechol (not shown).

Two observations indicated that the fluorescence signal from CTC-loaded acini was emitted largely from probe molecules incorporated into intracellular membranes which bind Ca^{2+} and Mg^{2+}. First, fluorescence microscopy showed that CTC fluorescence in acinar cells had a distribution similar to that of intracellular membranes[13]. Second, the fluorescence excitation spectrum of CTC in acinar cells was like that of CTC complexed with divalent cations in an amphipathic environment (λ_{max} = 386–400 nm) but unlike that of free CTC (λ_{max} = 382 nm) or of CTC complexed with divalent cations in aqueous solutions. In this environment, CTC fluorescence could be decreased by dissociation of its divalent cation complexes or by subsequent partitioning of the probe out of the membrane. We have detected, in fact, an increased exit of CTC from pancreatic acini during stimulation as well as the rapid decrease in fluorescence. This exit was not due to loss of CTC associated with contents of secretory granules since doses of bethanechol that elicited maximal amylase release produced fluorescence changes that were only 30% of maximum. Furthermore, fluorescence microscopy showed that secretory granule contents do not fluoresce[13]. Rather, studies with isolated mitochondria have indicated that complex dissociation and probe

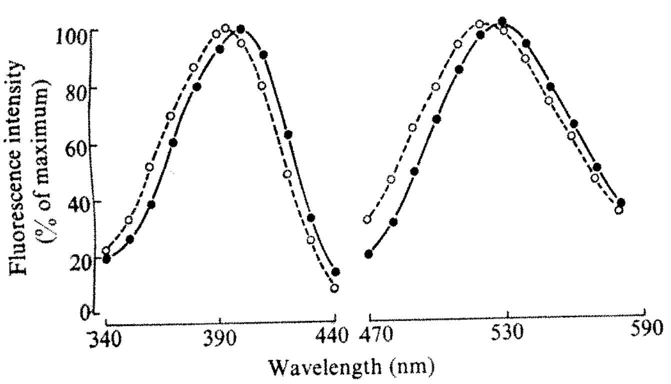

Fig. 3 Excitation and emission spectra of acinar cell-associated CTC immediately before (●) and 9 min after (○) bethanechol stimulation. Pancreatic acini were loaded with CTC, and their fluorescence monitored as described in Fig. 1. Spectra were corrected by subtracting the light scattering of an acini suspension not treated with CTC, then normalised to more clearly demonstrate the spectral shifts. Fluorescence intensity of maxima before and after stimulation were 70 and 45 units respectively. These spectral shifts were observed in each of five experiments with bethanechol and in four experiments using caerulein.

exit both occur when divalent cations dissociate from membranes containing CTC[10]. Thus, a reasonable explanation for the bethanechol-induced decrease in fluorescence is that Ca^{2+} or Mg^{2+} is released from some intracellular membrane.

Since Ca^{2+} and Mg^{2+} complexes of CTC can be distinguished by their excitation and emission spectra, we decided to record these spectra from CTC-loaded acini before and after stimulation. Normalised spectra in Fig. 3 show that before stimulation excitation and emission maxima were at 399 nm and 526 nm respectively. After stimulation these maxima were shifted to 393 nm and 520 nm. For comparison, model studies were done using 10^{-5} M CTC in 90% methanol-10% 10 mM Tris-HCl, *p*H 7.4, an amphipathic solvent in which the Ca^{2+} and Mg^{2+} complexes of CTC exhibit spectra similar to those in membrane environments[10]. In this slovent, Ca^{2+}–CTC exhibited excitation and emission maxima at 398 nm and 529 nm respectively, while these maxima for Mg^{2+}–CTC were at 386 nm and 519 nm. Thus, spectra from CTC-loaded acini indicated that CTC was complexed largely with Ca^{2+} before stimulation and largely with Mg^{2+} after stimulation. We concluded that the bethanechol-induced fluorescence decrease involved a preferential loss of signal from CTC complexed with Ca^{2+} in a membrane environment.

An intensity decrease accompanied by similar spectral shifts was also observed during stimulation by caerulein, a polypeptide containing the active site of CCK-PZ. The decrease in CTC fluorescence induced by bethanechol was blocked by atropine whereas that induced by caerulein was not. The fluorescence decrease in response to either secretagogue could be observed in the absence of extracellular calcium which was also the case for amylase release[2].

In control experiments, acini exhibited no changes in fluorescence intensity on stimulation when loaded with 1-anilino-8-naphthalenesulphonate (ANS) or oxytetracycline,-fluorescence probes that are not sensitive to membrane-bound divalent cations.

In view of the known sensitivity of chlorotetracycline fluorescence to membrane-bound divalent cations[8-12], these data are direct evidence for a release of calcium from intracellular membranes during stimulus–secretion coupling in pancreatic acinar cells. We are currently using chlorotetracycline to study the site and mechanism of this calcium release.

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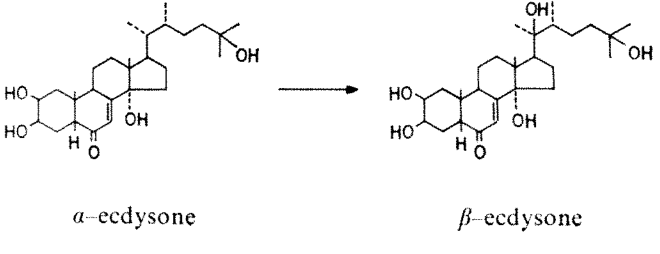
Evidence for an α-ecdysone cytochrome P-450 mixed function oxidase in insect fat body mitochondria

IT is now evident that α-ecdysone, a polyhydroxylated steroid, is synthesised and secreted by the insect prothoracic (ecdysial) glands[1-3]. β-ecdysone (20-hydroxy-α-ecdysone) seems, however, to have the major role in eliciting moulting during the post-embryonic development of insects[4]. If α-ecdysone functions as a pro(pre)-hormone as has been suggested[1,4], then it must undergo hydroxylation at C-20 of the side chain to form the moulting hormone, β-ecdysone. This conversion of α- to β-ecdysone is carried out by a hydroxylase which is located in tissues peripheral to the prothoracic glands[1,5,6]. Little is known about the subcellular distribution of this enzyme complex and there is virtually no information regarding the type of enzyme complex responsible for the oxygenation of steroids in insects in general. Using the vertebrate mixed function oxidase system as a model, we have investigated the subcellular distribution and enzymatic characteristics of the insect C-20 hydroxylase in last instar larvae of the tobacco hornworm, *Manduca sexta.*

In vertebrates, steroid hormone hydroxylations are catalysed by hydroxylase systems possessing a terminal cytochrome P-450 (refs 7 and 8). These enzyme complexes, termed mixed function oxidases or mono-oxygenases, incorporate one atom of molecular oxygen into substrate while reducing the other

Fig. 1 Stoichiometry of a cytochrome P-450 mixed function oxidase type reaction and the C-20 hydroxylation of α- to β-ecdysone.

$$\mathrm{RH + NADPH + H^{+} + O_2 \longrightarrow ROH + NADP^{+} + H_2O}$$



atom to water (Fig. 1). These P-450 mixed function oxidases are located in mitochondria and/or microsomes, are NADPH dependent and exhibit a characteristic CO difference spectrum absorption maximum at A_{450}. Since the C-20 hydroxylation of α-ecdysone in insects has characteristics in common with the P-450 mixed function oxidases mediating the hydroxylation of vertebrate steroid hormones (Fig. 1), it is reasonable to suggest that oxygenation of α-ecdysone is carried out by a similar enzyme complex.

C-20 hydroxylase activity was examined in whole tissues and homogenates incubated *in vitro* with 23,24-^{3}H-α-ecdysone (58 Ci mM^{-1}) to determine relative tissue hydroxylase activity. For both whole tissues and homogenates, the highest hydroxylase activity was associated with fat body, with lesser activity exhibited by Malpighian tubules, body wall, midgut, and perhaps salivary glands. C-20 hydroxylation of α-ecdysone did not occur in prothoracic glands, nerve cord, muscle, wing disks and haemolymph. Due to the high hydroxylase activity of the fat body, its abundance relative to other tissues, its accessibility and role as the major metabolic organ in insects, it was the tissue of choice for fractionation studies and biochemical characterisation of the C-20 hydroxylase.

Subcellular fractions of fat body homogenates were obtained by differential centrifugation and the C-20 hydroxylase activity determined (Table 1). The majority of the enzyme activity was associated with the mitochondrial fraction (64%), with reduced levels in the nuclear and microsomal fractions and none in the postmicrosomal supernatant. When the subcellular fractions were reconstituted, the C-20 hydroxylase activity for those pooled fractions approximated the sum of the activity for the individual fractions. This suggested that the enzyme activity associated with the nuclear and post-mitochondrial fractions may be a consequence of mitochondrial contamination. To verify this, the structural components of the subcellular fractions were examined by electron microscopy. The principal organelle observed in each fraction corresponded to what was expected, and the relative distribution of mitochondria correlated with the distribution of C-20 hydroxylase activity in the respective fractions. Mitochondrial and post-mitochondrial marker enzyme activities, cytochrome oxidase[9] and glucose-6-phosphatase[10] (possibly trehalose-6-phosphatase in insects[11]), respectively, were then monitored in each fraction and their distribution compared with that for the C-20 hydroxylase (Table 1). The percentage distribution of activity for the mitochondrial marker enzyme was consistent with that observed for the C-20 hydroxylase, whereas the post-mitochondrial enzyme activity was associated with the lowest C-20 hydroxylase activity. Therefore, on the basis of these data, it seems that the α-ecdysone C-20 hydroxylase is a mitochondrial enzyme and that the activity of the nuclear and microsomal fractions is due to mitochondrial contamination. This agrees with recent preliminary reports in which mitochondrial fractions from the midgut of *M. sexta*[12] and Malpighian tubules of the locust

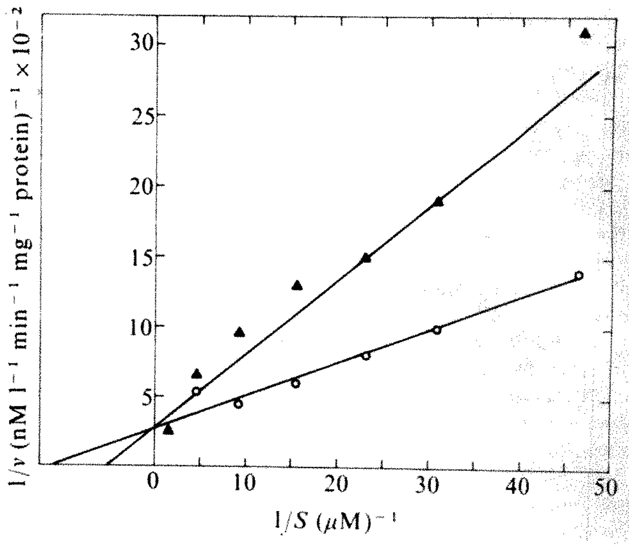


Fig. 2 Lineweaver–Burk plot for metyrapone inhibition of the fat body mitochondrial C-20 hydroxylase. The K_i for metyrapone (▲) is 3.9×10^{-6} M with a correlation coefficient of 0.997. The K_m for α-ecdysone is $1.6 \pm 0.7 \times 10^{-7}$ M (mean ± s.d. of 8 determinations). The K_m for α-ecdysone (○) in determining the metyrapone K_i was 9.0×10^{-8} M.

Table 1 Distribution of α-ecdysone C-20 hydroxylase, cytochrome oxidase and glucose-6-phosphatase activities in subcellular fractions of *M. sexta* larval fat body

Fraction	β-Ecdysone generated		Relative activity	
	ng	Relative activity	Cytochrome oxidase	Glucose-6-phosphatase
Whole homogenate	0.32	1.0	1.0	1.0
Nuclear (N)	0.07	0.23	0.28	0.12
Mitochondrial (M)	0.21	0.64	0.58	0.15
Postmitochondrial supernatant (PMS)	0.04	0.13	0.14	0.73
microsomal	0.04	0.13		0.34
postmicrosomal supernatant	0.0	0.0		0.39
N plus M	0.28	0.87		
N plus PMS	0.10	0.32		
M plus PMS	0.22	0.69		
N plus M plus PMS	0.31	0.97		

Fat body from day 1 fifth instar *M. sexta* larvae was homogenised (50 mg ml^{-1}) in 0.05 M $NaH_2PO_4 \cdot Na_2HPO_4$ buffer (*p*H 7.5) containing 0.25 M sucrose at 0 °C. Subcellular fractions were obtained by differential centrifugation at –5 °C: nuclear, 800*g* for 10 min; mitochondrial, 12,000*g* for 10 min; microsomal, 105,000*g* for 1 h. Pellets were washed once and resuspended in the same buffer to the original concentration of 50 mg tissue equivalents ml^{-1}. For the C-20 hydroxylase assay, 0.05 ml subcellular fraction was incubated with 0.05 ml (0.05 M $NaH_2PO_4 \cdot Na_2HPO_4$ buffer (*p*H 7.5) containing 1.5×10^5 d.p.m. 23,24-^{3}H-α-ecdysone (58 Ci mM^{-1}, 1.2×10^{-8} M) and 37.5 μg NADPH (4.0×10^{-4} M) for 30 min at 30 °C. All assays were run in duplicate. Ethanol (2.0 ml) was added to terminate the reactions. Terminated assays were centrifuged (10,000*g* for 10 min) and 200 μl of the supernatant (1.5×10^4 d.p.m.) plus 1.0 μg carrier α- and β-ecdysone were streaked on thin layer plates (EM, silica gel 60, F-254) and developed in a solvent system of chloroform: 95% ethanol, 80: 20 (v/v). Following visualisation of the α- and β-ecdysone bands under ultraviolet light, these zones were scraped and counted. The efficiency of separation was greater than 94 ± 2.5% that obtained using high-pressure liquid chromatography[3]. Cytochrome oxidase[9] and glucose-6-phosphatase[10] activities, as well as protein concentrations[18], were determined as described previously. Glucose-6-phosphatase has not been demonstrated to be a microsomal marker enzyme in insects but has been shown to be associated with postmitochondrial fractions, and may actually be trehalose-6-phosphatase, which is a post-mitochondrial enzyme in insects[11]. Since trace amounts of substrate were used for the C-20 hydroxylase assay, potential compartmentalisation of endogenous α-ecdysone into selected subcellular fractions could have reduced the apparent hydroxylase activity in these fractions. Therefore, the endogenous α-ecdysone in each fraction was determined by radioimmunoassay and was observed to be the same for all fractions (concentrations ~ 2×10^{-2} of the ^{3}H-α-ecdysone used for the assay).

Schistocerca gregaria (H. Rees, personal communication) exhibited C-20 hydroxylase activity.

To characterise the C-20 hydroxylase as a mixed function oxidase, it was necessary to demonstrate that the hydroxylation was enzymatically mediated. Enzyme activity profiles under varying regimens of temperature and pH were obtained, with the observed optima being 30 °C and pH 7.5, respectively. The reaction was not affected by most monovalent and divalent cations, but Mg^{2+} ions inhibited hydroxylation (up to 25%) at concentrations as low as 10 mM. Time course analysis of substrate conversion revealed a linear response up to 60 min with a plateau obtained at 90 min. When increasing concentrations of mitochondria were incubated with fixed levels of substrate, proportionate increases in the hydroxylation of substrate to product were observed. Hyperbolic saturation kinetics were obtained when increasing levels of substrate were incubated with fixed concentrations of mitochondria. An apparent K_m of $1.6 \pm 0.7 \times 10^{-7}$ M (mean ± s.d.) was derived from Lineweaver–Burk plots obtained by linear regression analysis of saturation kinetics data (Fig. 2). Using an *in vivo* kinetics system, a similar K_m has been reported for the C-20 hydroxylase of the fly *Calliphora stygia*[13]. Chemical identification of β-ecdysone as the product of this putative C-20 hydroxylase was attained by acetylation finger printing of labelled product[3] and ultimately by mass spectrometry of 1.0 µg unlabelled material (m/e 462, 444, 426, 408, 345 and 328).

Table 2 Effect of various co-factors and gases on the oxygenation of α-ecdysone by fat body mitochondrial C-20 hydroxylase

Co-factor	Relative C-20 hydroxylase activity
NADPH	1.0
NADP	0.07
NADH	0.27
NAD	0.01
ATP	0.05
ADP	0.03
NADPH-generating systems:	
Glucose-6-phosphate dehydrogenase	1.0*
Krebs cycle intermediates:	†
Malate	0.13
Malate + NADP	0.59
Fumarate + NADP	0.11
Isocitrate + NADP	0.12
Gases	
Air	1.0
Ar	0.97
CO_2	0.05‡
CO	0.38
N_2	0.95
O_2	0.86

The method for assaying C-20 hydroxylase activity was as in Table 1. A dose response was determined for all cofactors at concentrations from 2.0×10^{-7} to 4.0×10^{-4} M. For NADPH and NADH, C-20 hydroxylase activity was dose dependent with a $V_{max/2}$ for NADPH of 6.0×10^{-6} M. Maximum oxygenation with NADH was not obtained at concentrations up to 4.0×10^{-4} M. For the rest of the cofactors, hydroxylase activity did not increase significantly above background. Gases were introduced to the standard assay system by bubbling at a flow rate of 35 ml min^{-1} for 2 min. Following the introduction of gases and incubation, O_2 was present at a concentration of approximately 60 nmol ml^{-1}, which was 25% of the O_2 concentration of controls incubated in the absence of added gas. Stoichiometrically, this is a 5.4×10^3 excess of O_2 for the C-20 hydroxylation of α-ecdysone and precludes oxygen depletion as an explanation for the observed changes in C-20 hydroxylase activity in these gas studies.

*A glucose-6-phosphate dehydrogenase NADPH-generating system provided maximal C-20 hydroxylase activity at 4.0×10^{-6} M NADPH generated.

†Krebs cycle intermediates (2×10^{-2} M) were used to generate NADPH from exogenous NADP (4×10^{-4} M, equivalent to the NADPH in the hydroxylase assay). The ultimate effect of the Krebs cycle intermediates is apparently to provide an increased reduced cofactor (NADPH) pool for hydroxylase activity, as evidenced by the requirement for NADP with the Krebs cycle intermediates. Succinate, citrate, α-ketoglutarate and oxaloacetate in the presence of NADP did not increase hydroxylase activity above that observed for NADP alone.

‡Low conversion in the presence of CO_2 was probably due to acidification of the assay mixture by CO_2 to pH 6.2, which is below the minimum pH (6.5) for preservation of C-20 hydroxylase activity.

Substrate specificity of the C-20 hydroxylase is at present being assessed using a variety of steroids and ecdysone analogues. Preliminary results suggest that there is a high degree of specificity which depends on the number and position of hydroxyl groups, and more importantly, on the stereochemistry of the AB ring and side chain.

To demonstrate that the mitochondrial α-ecdysone C-20 hydroxylase is a cytochrome P-450 mixed function oxidase, the following criteria were used: dependence on NADPH (using an exogenous NADPH or NADPH generated by a glucose-6-phosphate dehydrogenase system[14] or NADPH generated by Krebs cycle intermediates[15]); inhibition by CO[7,8], metyrapone (Metopiron)[16], aminoglutethimide (Elipten)[16] and p-chloromercuribenzoate[14]; and a CO difference spectrum[17]. The C-20 hydroxylase activity was dependent on NADPH or NADPH generating systems as electron donors, with NADH being only about 25% as effective (Table 2). The C-20 oxygenation of α-ecdysone was efficiently inhibited by CO (Table 2), p-chloromercuribenzoate ($I_{50} = 5.0 \times 10^{-7}$ M), aminoglutethimide ($I_{50} = 1.0 \times 10^{-5}$ M) and metyrapone ($I_{50} = 5.0 \times 10^{-6}$ M). Metyrapone acts as a competitive inhibitor with an apparent K_i of 3.9×10^{-6} M (Fig. 2). The CO difference spectrum obtained for the CO-bound sodium dithionite-reduced mitochondria was similar to those obtained from vertebrates with an absorption maximum at A_{450} (Fig. 3).

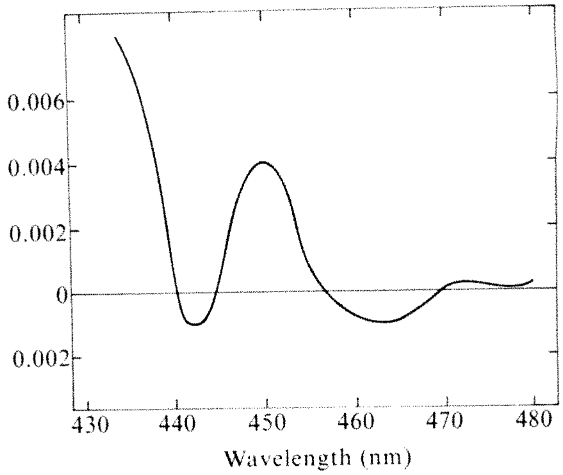


Fig. 3 A CO difference spectrum of sodium dithionite-reduced fat body mitochondria. The sample cell contained a suspension of dithionite-reduced mitochondria saturated with CO, whereas the reference cell contained only dithionite reduced mitochondria. The difference spectrum was obtained using a Cary 14 spectrophotometer equipped with a transmission scatter attachment.

The above studies demonstrate that the α-ecdysone C-20 hydroxylase is located in the insect fat body mitochondria and that cytochrome P-450 is probably the oxygen activator for the oxygenation of α- to β-ecdysone. This is the first report implicating a cytochrome P-450 mixed function oxidase in the oxygenation of steroids in insects, and in arthropods in general.

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Potential-dependent transition temperature of ionic channels induced by glutamate in locust muscle

RECENT work has emphasised the important role which the state of the membrane has in the function of its transport and enzyme systems[1,2]. At various synapses the action of transmitter substances is accompanied by 'membrane noise' associated with the opening and closing of ionic channels. Analysis of this noise gives an estimate of the mean conductance and lifetime of the single open channel[3–9]. In locust muscle we have found that the mean lifetime (τ) of channels, activated either by the natural transmitter or by externally-applied glutamate, is prolonged as the temperature is lowered, while the conductance (γ) of the single channel is decreased. The dependence of γ and τ on temperature shows a change in slope at a temperature which itself depends on the membrane potential. These results are possibly due to a potential-dependent phase transition in the membrane around the channel causing an abrupt change in the operation of the gating molecule and conductance-determining portion of the channel.

Distal fibres of the metathoracic extensor tibiae muscle[10] of the locust (*Schistocerca gregaria*) were voltage-clamped using two intracellular microelectrodes. L-glutamate was applied ionophoretically from a micropipette to a junctional site[7,11]. The bathing medium was as previously described and contained increased Mg^{2+} and decreased Ca^{2+} in experiments where the nerve was stimulated. Bath temperature was controlled with a Peltier cooling device and monitored with a thermistor placed near the muscle bundle under investigation. Bath reference electrodes were isolated through agar bridges to reduce temperature-induced junction potentials. Temperature readings were accurate to within ±0.5 °C.

The energy spectrum of the current noise obtained during steady application of glutamate to junctional receptors in locust muscle results from statistical fluctuations in the opening of ionic channels, and agrees well with a first order process which follows the relationship[7]

$$S(f) = \frac{2\gamma\mu_g/\alpha}{1 + (2\pi f/\alpha)^2} \tag{1}$$

characterised by the independent parameters γ and α. Under a specific hypothesis for the operation of the glutamate-sensitive receptor complex[7], similar to that suggested for acetylcholine receptors in vertebrate muscle[3,4,12], γ and α are respectively the conductance and closing rate constant of the single ionic channel. The mean life-time of the open state is given by $\tau = 1/\alpha$.

For a given mean conductance (μ_g) lowering the temperature prolongs the mean life-time (τ) of the open channel (seen as a shift in the cut-off frequency of the noise spectrum) and reduces the variance of the noise, by decreasing the conductance of the single channel (seen as a reduction in the zero frequency asymptote, $S(0)$, of the spectrum). Apart from altering γ and α, changing the temperature did not alter the spectral relationship (equation 1) over the range studied (2–25 °C).

Figure 1 shows the temperature-dependence of γ (the single-channel conductance), and α (the rate constant for channel closing) at a fixed membrane potential. γ and α decrease exponentially with the inverse of the absolute temperature (°K) at all temperatures studied but, at about 6 °C the slope of the

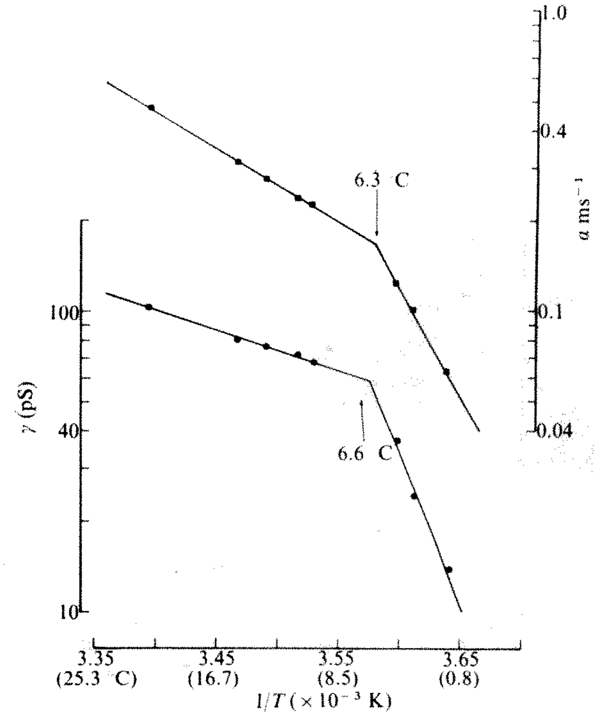

Fig. 1 Temperature dependence of the glutamate-induced single-channel characteristics. The channel closing rate constant (■) α (ms^{-1}) and the single channel conductance (●) γ (pS) are plotted semilogarithmically against T^{-1} (Kelvin). Temperatures in °C are given in parentheses. Values were obtained during steady ionophoretic application of glutamate by fitting the membrane current fluctuation spectra to the relationship in equation (1). Background noise spectra were subtracted before fitting. Transition temperature for α is 6.3 °C and for γ is 6.6 °C, for a membrane potential of −100 mV. The agreement between the data and the solid lines suggests that α and γ behave according to a reaction scheme in which the apparent activation energy increases sharply below the transition temperature. Data is from six voltage-clamped fibres. Each point represents the spectral parameters of the average spectra from 20 noise segments each digitalised at 2,048 Hz in 1,024 point segments over 0.5 s. Spectral estimates were calculated using an F.F.T. algorithm.

relationship changes abruptly. The values for γ and α may be fitted to the relation depicted by the solid lines in Fig. 1, which represent the behaviour expected of first-order rate processes. For α, this hypothesis is governed by the relationship[12]

$$\alpha = \hat{\alpha} e^{-E_\alpha/RT} \quad (2)$$

where E_α is the energy required for movement of the gating molecule where α is constant, T is absolute temperature in °K and R is the gas constant. The lines drawn for α in Fig. 1 show the relationship for $T > 6$ °C, where $\alpha_1 = 1.15\times10^8$ ms^{-1}, $E_{\alpha2} = 11.4$ kcal mol^{-1} and for $T < 6$ °C, $\alpha_2 = 1.37\times10^{25}$ ms^{-1}, $E_{\alpha2} = 33.3$ kcal mol^{-1}.

If the single-channel conductance is envisaged as being determined by the rate of transfer of ions, ν, across an energy barrier, E_γ, in the postsynaptic membrane, then the resulting first order rate process may be represented by

$$\nu = \hat{\nu} e^{E_\gamma/RT} \quad (3)$$

which yields the unitary conductance

$$\gamma = \nu ZF/(V - V_{eq})A \quad (4)$$

where Z is valence, F is Faraday's constant and A is Avogadro's number. The lines drawn for γ in Fig. 1 show the relationship for $T > 6$ °C where $E_{\gamma1} = 6.12$ kcal mol^{-1} and for $T < 6$ °C, $E_{\gamma2} = 46.5$ kcal mol^{-1}. The transition temperature for α is 6.3 °C (at $V_m = -100$ mV) and for γ is 6.6 °C ($V_m = -100$ mV). Transition temperature values for α and γ are practically the same within the accuracy of our measurements.

At acetylcholine receptors in frog muscle[4] and at glutamate receptors in locust muscle[7], the postsynaptic excitatory junctional current (e.j.c.) evoked by nerve stimulation has an exponential decay with a time constant ($\tau_{e.j.c.}$) identical to that which characterises the $1/f^2$ component of the current noise spectrum; $\tau_{e.j.c.} = 1/2\pi f_c = \alpha/2\pi$, where f_c is the half-power frequency of the spectrum. This allowed us to investigate the temperature dependence of α for channels activated by the natural transmitter. This technique was also used to study more fully the behaviour of the rate constant close to the point of transition, and particularly its dependence on membrane potential.

Figure 2 shows a semilogarithmic plot of $\tau_{e.j.c.}^{-1}$ as a function of T^{-1} at two membrane potentials. The relation follows two straight lines at each potential without obvious curvature near the critical temperature. This may suggest a discrete change in a single rate limiting energy barrier rather than a dual process with different temperature dependencies, or a single kinetic scheme in which the activation energy is a continuous function of temperature. The results fit well the relationship $\tau = \tau e^{(E_a + \eta(T))/RT}$, which is predicted from an energy barrier model for the closing of channel gates (unpublished) in which the free energy change of the system is coupled to the electric field through a temperature-dependent dipole (η). Values for the apparent activation energies and transition temperatures are given in Table 1. As is seen in Fig. 2, the transition temperature depends on membrane potential in such a way that depolarisation causes a shift in transition temperature to higher values. Transition temperature seemed to show a linear dependence on membrane potential.

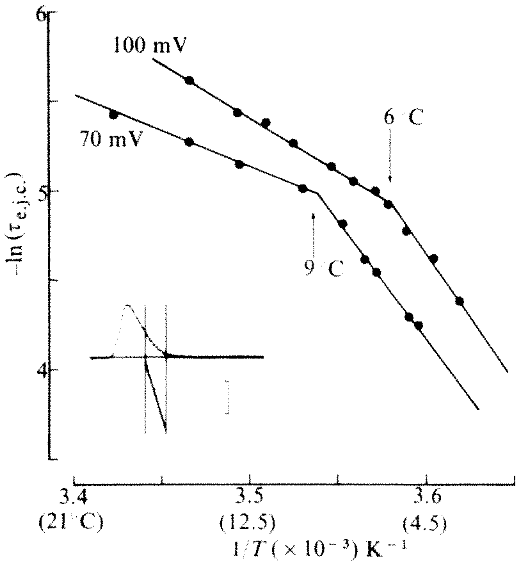

Fig. 2 Arrhenius plot of temperature dependence of the time constant of decay of nerve-evoked e.j.c.s in two muscle fibres clamped at −100 mV and −70 mV respectively. As shown in the inset, digitalised e.j.c.s were plotted semilogarithmically and a least squares line fitted to the decay phase gave $\tau_{e.j.c.}$ (calibration bar 80 nA; e.j.c. is 70 ms full sweep; V_m, 100 mV; T, 20 °C). Each point on the graph represents at least 50 such determinations. In all cases the standard error of the mean is smaller than the symbol. Transition temperatures obtained from the intercepts 6.1 °C at V_m = 100 mV and 9.1 °C at V_m = 70mV. Fibre input resistances were 2.1 MΩ (V_m, 100 mV) and 5.2 MΩ(V_m, 70 mV). Clamp efficiency was better than 99.6% at 0 Hz and better than 90% at 1.6 kHz. Temperatures (°C) are given in parentheses.

We conclude that the apparent activation energies of the rate processes characteristic for the synaptic gate and the conductance-determining portion of the ion channel undergo a discrete change in value at a transition temperature which depends on membrane potential.

One interpretation of the break in the Arrhenius plots shown in Figs 1 and 2, is that it reflects a conformational change in the receptor—and associated ionic channel—similar to that observed in some proteins[13]. Another possibility is that the break is a consequence of a lipid phase transition in the membrane[1,2]. A change in the state of membrane lipids has already been invoked to explain the effects of alcohols on miniature endplate currents in toad muscle[14]. But, the possibility that the fluidity of the lipids associated with the receptors may influence their function, should be considered only as a speculation until direct measurements are made on the nature and state of the lipids surrounding the receptors.

If the break in the temperature dependence of α and γ is due to a lipid phase transition, then several questions arise. For

Table 1 Apparent activation energies (E_{a1}: above transition temperatures; E_{a2}: below transition temperatures) for the rate constant for channel closing α and for the rate constant for the transmembrane flow of ions ν

	Rate constant	V_m (mV)	E_{a1} (kcal mol^{-1})	Q_{10}	E_{a2} (kcal mol^{-1})	Q_{10}	E_{a1}/E_{a2}	Transition temperature (° C)
Glutamate	α	−100	11.4	1.8	33.3	6.3	1/2.9	6.3
	ν	−100	6.1	1.2	46.5	7.2	1/7.6	6.6
Transmitter	α	− 70	8.2	1.7	25.9	5.4	1/3.2	9.1
	α	−100	11.9	2.2	28.1	6.6	1/2.4	6.1

Values are averages based on fitting the relationships $\alpha = \alpha e^{E_a/RT}$ and $\nu = \nu e^{E_\gamma/RT}$ to the data for $\alpha(V_m,T)$ and $\gamma(T)$ respectively. Values obtained in response to glutamate or in response to neurally released transmitter as indicated.

instance, in cell membranes the transition usually occurs over a broad change in temperature and the Arrhenius plot may show two breaks and three segments: one corresponding to the transition range, and one above and one below the transition. Our plots seem to have only two segments. This could indicate that the transition occurs over a narrow range in temperature, perhaps as a result of the close packing and relative immobility of the receptors which may be anchored in the synaptic membrane. Alternatively, the lower end of the transition may not have been reached.

The similarity of the transition temperatures for single channel conductance (γ) and for the rate constant of channel closing (α) may indicate that both processes are affected by the same mechanism. Perhaps the glutamate recognition site of the receptor, and that part of the receptor which contains the main energy barrier for ion translocation, lie close together and penetrate well into the membrane so that both portions may be altered by changes in lipid structure. In embryonic chick muscle[9] and at mammalian endplates[15], a break has been observed in the relationship between temperature and channel conductance, but not in that between temperature and lifetime of the channels induced by acetylcholine. The reason for this apparently different behaviour of glutamate and acetylcholine receptors is not yet clear. Perhaps the recognition site of the acetylcholine receptor lies more in the aqueous external face of the membrane, and relatively distant from the membrane-embedded portion which controls the ionic flux; in which case a lipid phase transition may not exert an important influence on the drug–receptor kinetics.

If the transition we described results from a phase change in the lipid associated with the receptor, then the voltage dependence of the transition could result from a coupling of the membrane electric field to the charge distribution in the lipid component.

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Are cell affinity relationships for insect epidermal tissues evidence for combinatorial control?

THE segregation of determined, yet undifferentiated, cells in aggregates consisting of cells from various vertebrate embryonic structures represents a high degree of specificity in cellular recognition[1–3]. Steinberg's differential adhesiveness model[2] has been particularly successful in accounting for this sorting out of cells in terms of differences in their adhesiveness. Although arranged in monolayers, insect epidermal cells seem to behave similarly when their normal spatial organisation is disrupted. The regrouping of disaggregated *Drosophila* imaginal disk cells according to epidermal type and the failure of grafts from one epidermis to form stable contacts with cells of another epidermal tissue have both been attributed to sorting out of unlike cells[4,5]. Yet some insect epidermal cell types fail to segregate completely from one another; even when disaggregated and mixed, cells from genetically marked leg and antenna disks are known to form some integrated patterns[6]. It is possible that different degrees of adhesiveness exist for various combinations of two different epidermal types, the adhesive properties not being uniquely specified for each tissue. With this possibility in mind, exchanges between grafts and hosts of various pupal epithelia of the moth, *Manduca sexta*, were performed. The affinity relationships observed reflected the existence of covert cell surface differences in these epidermal populations whose component cells at the time of grafting are determined but undifferentiated. The findings suggest that the cell surface properties are not tissue type specific but are shared to various degrees by the different epidermal tissues.

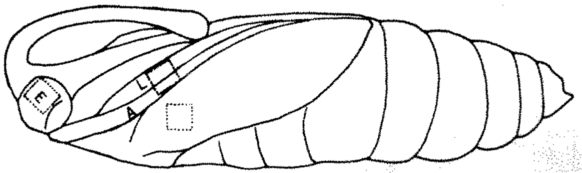

Fig. 1 The tissue regions used for grafting in the study of inter-tissue affinities. Dorsal is to the bottom. Four different epidermal tissues were selected: wing (W), eye (E), antenna (A), and mesothoracic leg (L). The graft sizes varied slightly but were usually 1.5 × 1.5 mm, each consisting of approximately 6,000 cells. All grafts were removed from recently pupated animals (6–15 h after pupation); at this stage the cells showed no evident morphological differences in the light microscope. Because of the proximity of antenna and leg epidermis to each other, these two epithelia were removed and transferred as a single graft unit. In all transpositions (except between leg and antenna) the original orientations of the grafts were retained. In the contralateral exchanges between antenna and leg, which were necessitated by the fact that the two tissues were handled only in combination, the dorsoventral axes of these tissues were reversed without concomitant reversal of their proximo-distal axes. Therefore, the possibility existed that misalignment of the dorsoventral axes of host and graft influenced their mutual affinities. But, in control experiments involving similar contralateral exchanges between either an antennal pair or a leg pair, differences in epidermal properties were never manifested.

Animals for experiments were reared, selected, and treated as described in Nardi and Kafatos[7]. The pupal tissue regions chosen for the study are shown in Fig. 1. Contact between two different tissues was scored in animals just before the time of adult emergence from the pupal cuticle (3 weeks after pupation) and varied from contact involving little or no decrease in original graft perimeter to the formation of completely free, differentiated graft vesicles. By carefully removing the overlying pupal cuticle from the grafting sites, all graft tissues were recovered; otherwise unattached vesicles would have been lost during adult emergence. Only the presence or absence of contact between host and graft was scored, and the results were expressed as fractions of grafts that maintained cell–cell contact with host tissues. Because of the ease in distinguishing free vesicles from attached graft tissues under a dissecting microscope, ambiguities in scoring were avoided. Figure 2 shows typical grafts that were scored as having maintained cell contact with the host.

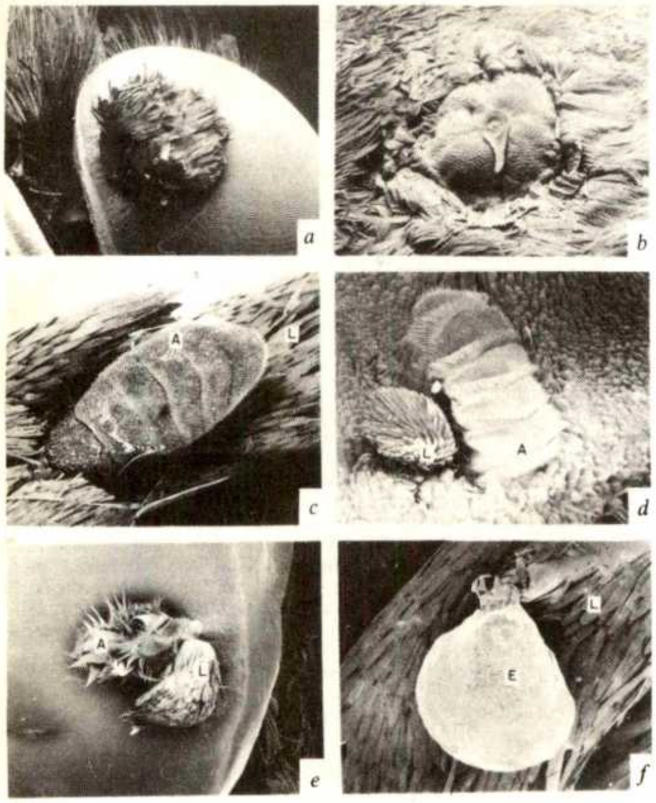

Fig. 2 Scanning electron microscope photographs of representative grafting results. *a*, Extensive contact exists between the wing cells and the eye epidermis (×28.5). *b*, Eye tissue transposed to the wing (×42). *c*, Female antenna (A) tissue adhering to the leg (×63). *d*, Leg (L) tissue and male antenna epidermis (A) tenuously attached to the wing surface (×45). *e*, Male antenna epidermis and leg epidermis grafted to eye. Only the antenna tissue is attached to eye; the leg epidermis is attached to the antenna (×39.5). *f*, An inverted eye vesicle attached to the leg (×120).

No evidence of vesicle formation was found for grafts exchanged within a given epithelium (Table 1); this was true whether grafts were transferred between different positions in the same epidermis or exchanged between corresponding sites of two pupae. Moreover, a particular epithelial tissue shows no consistent tendency either to fail or succeed in establishing contact with different epithelial cell populations. These facts argue against the possibility that the observations reflect grafting artefacts rather than cell surface differences.

Table 1 Fraction of grafts which form stable contacts with host tissues

Graft	Host	No. of exchanges	No. of adhering grafts	Fraction of grafts adhering (affinity value)	Significance level of differences in affinity values for a given exchange between two epidermal types
W	W	34	34	1.0	—
L	L	34	34	1.0	—
A	A	34	34	1.0	—
E	E	34	34	1.0	—
W	L	35	11	0.3	$P<0.03$
L	W	35	21	0.6	
W	A	35	14	0.4	$P<0.01$
A	W	35	32	0.9	
W	E	35	8	0.2	$P<0.02$
E	W	35	19	0.5	
L	A	46	17	0.4	$P<0.01$
A	L	46	46	1.0	
E	L	33	1	0.0	$P>0.10$
L	E	33	1	0.0	
E	A	33	7	0.2	$P<0.01$
A	E	33	23	0.7	

Several notable features emerge from Table 1. First, there is considerable disparity in affinities. For example, values range between the extreme values of 1.0 for antennal grafts transposed to leg epidermis and 0.0 for leg epidermis transposed to eye tissue ($P < 0.01$). A given graft does not show the same affinity for all the host sites examined. This is clear for exchanges of L→E and L→W, these values being 0.0 and 0.6, respectively ($P < 0.01$). Second, in a reciprocal exchange the affinity value is a function of which tissue is the graft and which is the host. For most of the pairs listed, the two affinity measures are not equivalent (with the exception of the eye, leg exchanges). This disparity is well illustrated by a comparison of the 0.4 affinity value for L→A to the value of 1.0 for A→L ($P < 0.01$). Other reciprocal exchanges show statistically significant differences in affinity values. Third, evidently no correlation exists between affinity and spatial arrangement of *Manduca* tissues. The distance separating the eye from the leg is no greater than that separating the eye from the wing region; yet the fractional values for the E→L and E→W exchanges differ by almost a factor of ten.

The affinity asymmetry for two given tissues is most readily accounted for by a model which deals with pairs of tissues that are either at configurational equilibrium or that are approaching it through minimisation of their surface free energies[2,7]. In applying the model to an exchange between A and B tissues whose cells are arranged in monolayers, B graft cells, after a given time period, will show more extensive contact with A host cells than that displayed by A grafts with B host tissue provided that certain A–B relationships hold true, that is, the cohesiveness of A cells exceeds the cohesiveness of B cells. This situation is analogous to the asymmetrical, albeit three-dimensional, interaction of two immiscible liquids A and B, one of which exists either as a liquid drop or a film on the surface of a liquid substrate. A drop of A will form as long as the sum of the surface energy for A and the surface energy of the A–B interface exceeds that for substrate B; but if the surface energy of the substrate A exceeds the total surface energy for drop B and the A–B interface, then B augments its contact with the substrate. In speaking of surface energy for monolayers of cells, we are dealing with the energy for those lateral cell surfaces which establish contact with adjacent cells. This energy is a reflection of the cells' cohesiveness.

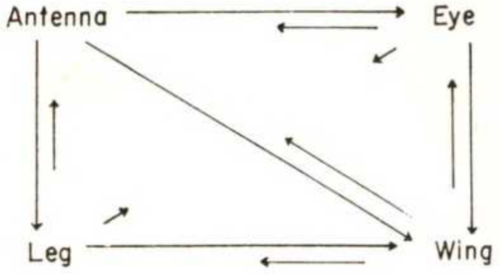

Fig. 3 Mutual affinities of various *Manduca* epidermal tissues. The unidirectional arrows between two epithelia point from the graft to the host tissue; and the relative magnitudes of the arrows for a given exchange reflect relative affinities for that exchange.

According to the combinatorial model of Kauffman which deals with the phenomena of determination and transdetermination in *Drosophila*, the global directionality that is manifested by the transdetermination pathway and the affinity relationships in Fig. 3 (that is, the prevailing orientation for every exchange is towards wing) may indicate the existence of underlying bistable control systems[8]. Another model involving binary developmental decisions—the selector gene model—is based on genetic analysis of *Drosophila* development and was independently developed by Garcia-Bellido[9]. In this combinatorial model, selector genes comprise that set of genes controlling developmental pathways and determination. Of major relevance to the study discussed in this report is that work with one such gene, the wild-type allele of the *engrailed* locus, has suggested that the activity of selector genes supplies cells with distinctive surface labels that govern cellular recognition[10].

Although the nature of the relationship between determination and the mutual adhesiveness of different epidermal cells remains obscure, the striking correspondence between these two phenomena suggests that, regardless of the location of the determinative factors, the cell surface properties are probably closely associated with decisions involving cell determination.

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Control of induction of tubulin synthesis in *Chlamydomonas reinhardi*

REMOVAL of the flagella from the biflagellate alga *Chlamydomonas reinhardi* results in the rapid production of new flagella[1–3]. Flagellar regeneration is 80–85% complete within 60 min (Fig. 1). Indirect evidence obtained from *in vitro* translation of polyribosomes and mRNA from deflagellated gametes[1] has indicated that flagellar regeneration is accompanied by a strong but transient induction of tubulin synthesis. Kinetic analysis showed that tubulin mRNA could be detected in polyribosomes in as little as 15 min after deflagellation. Since the cytoplasm contains a reserve of tubulin of sufficient size to supply at least one-third to one-half of the tubulin subunits required for flagellar reassembly[1,3], the rapid initiation of tubulin synthesis following deflagellation suggested the existence of a highly sensitive system for detecting and responding to the cell's anticipated need for additional tubulin. As an approach to seeking such a mechanism, we have carried out studies to determine if the depletion of tubulin reserves during flagellar outgrowth might provide the signal for the induction of tubulin synthesis. We present here two types of experimental evidence which clearly demonstrate that the induction of tubulin synthesis is independent of the utilisation of tubulin reserves during flagellar regeneration.

Comparison of the spectrum of proteins produced by control and deflagellated gametes during a 30 min pulse label with $^{35}S\text{-}H_2SO_4$ shows that deflagellation results in a marked induction of the α (56,000 molecular weight) and β (53,000 molecular weight) subunits of tubulin (see tracks 2 and 6, Fig. 2). Chloramphenicol is added during *in vivo* labelling to prevent the synthesis of the 55,000 molecular weight subunit of ribulose bisphosphate carboxylase (tracks 3 and 4) that would otherwise interfere with the analysis of tubulin synthesis (tracks 1 and 5 compared with 2 and 6). The magnitude of tubulin induction following deflagellation is indicated by densitometric analysis of the radioautographs presented in Fig. 3 (tracks 1–4). In the time intervals 15–45 min, 45–75 min, 105–135 min and 165–195 min, tubulin synthesis accounts for 18.1, 14.5, 7.7 and 3.2 of total cellular protein synthesis (4% has been subtracted from each value to compensate for 'background' incorporation in the 53–56,000 molecular weight region of gels containing proteins synthesised by non-deflagellated cells).

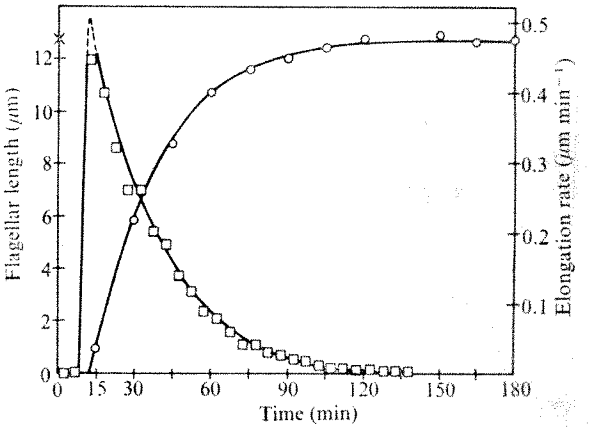

Fig. 1 Flagellar regeneration in gametes of *C. reinhardi*. Gametic cells were deflagellated by vigorously stirring cells at *p*H 4.7 for 1 min and then raising the *p*H of the medium to 7.0 (ref. 3). At various times after deflagellation, cells were fixed with 1% glutaraldehyde and 10 mM potassium phosphate (*p*H 6.8), and flagellar length measured as described previously[1]. ×, Initial flagellar length; ○, flagellar length; □, rate of flagellar elongation. For preparation of gametic cultures, synchronously grown vegetative cells of strain 137c near saturation density were transferred to a nitrogen deficient media, either medium 2-N (ref. 1) or medium 2-N without sulphate (designated medium V) in which an equivalent level of $MgCl_2$ was substituted for $MgSO_4$. If gametes were formed by overnight incubation in medium 2-N, they were rinsed and re-suspended in medium V 1 h before deflagellation (or before labelling with $^{35}S\text{-}H_2SO_4$).

The above *in vivo* labelling experiments provide a direct demonstration of the rapid and extensive nature of tubulin induction following flagellar excision. Additional experiments (not presented) have shown that the initiation of tubulin synthesis occurs between 12 and 15 min after deflagellation. Thus, if *de novo* synthesis of tubulin mRNA is required for tubulin induction, as suggested by the observation that tubulin mRNA is not detectable in non-deflagellated cells[1], then the time requirements for mRNA transcription, processing and transport[10], dictate that the signals required for the activation of tubulin genes must be generated by reactions intimately associated with the deflagellation event. Since flagellar regeneration is not observed until 10–12 min after deflagellation (Fig. 1), the kinetic data suggest that utilisation of tubulin subunits for flagellar outgrowth cannot serve as a feedback signal for the induction of tubulin synthesis. As a further, more direct, test of this question, we have treated cells with a variety of compounds that block flagellar regeneration to determine if these treatments have any effect on tubulin induction. Results presented in Fig. 3 show that treatment of cells with 1 mM isobutylmethylxanthine (IBMX) either 15 min before deflagellation (tracks 5–8) or immediately after deflagellation (tracks 9–12) has no effect on the early phases of tubulin induction even though IBMX, like other methylxanthines[11,12], completely blocks flagellar outgrowth. Treatment of deflagellated cells with colchicine, chloro-isopropyl-*n*-phenylcarbamate, 50 mM potassium phosphate (*p*H 6.8) and other agents that prevent flagellar regeneration, provide nearly identical results to those obtained with IBMX (not shown). These findings provide clear evidence that utilisation of tubulin reserves for flagellar outgrowth cannot serve as a signal for the initiation of tubulin synthesis.

Finally, it may be noted that although intracellular tubulin concentrations do not seem to control the early phases of tubulin synthesis, indirect evidence indicates that increasing reserve levels may govern tubulin production during the later stages of flagellar regeneration. A comparison of data in Figs 1 and 3 shows that relatively high levels of tubulin production continue well beyond the time when flagellar outgrowth is essentially complete and therefore suggests that tubulin reserves accumulate during the late stages of regeneration.

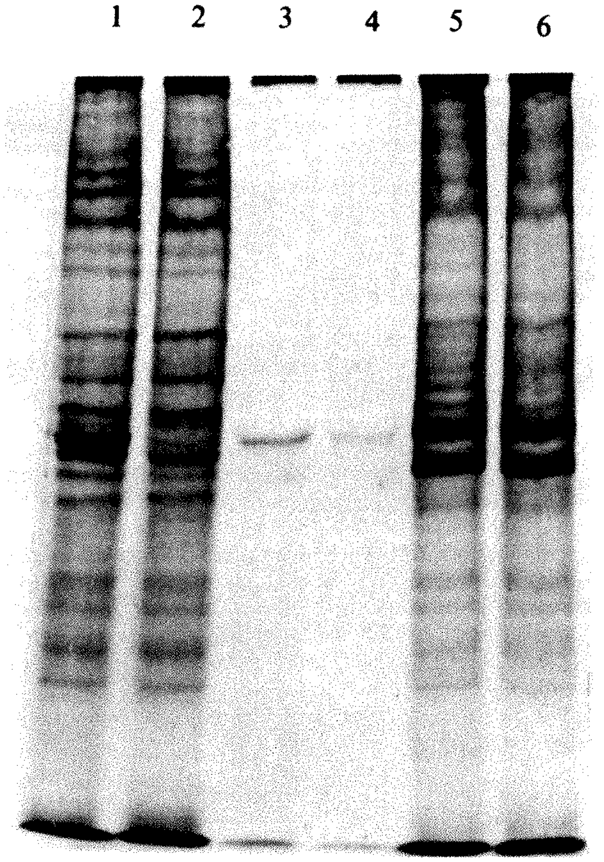

Fig. 2 Autoradiographs of SDS–polyacrylamide gels containing *in vivo* labelled proteins from deflagellated *Chlamydomonas* gametes. Cells were labelled with $^{35}S-H_2SO_4$ using modifications of procedures developed by Lefebvre and Rosenbaum (personal communication) and other laboratories[5–8]. Approximately 10^7 sulphate-starved cells in 0.5 ml were labelled for 30 min in 100 μCi carrier-free $^{35}S-H_2SO_4$. Non-deflagellated cells were incubated without protein synthesis inhibitors (track 1); with 100 μg ml^{-1} chloramphenicol to inhibit chloroplastic protein synthesis (track 2); with 10 μg ml^{-1} cycloheximide (track 3) or with 10^{-4} M 2-(4-methyl-2,6-dinitroanilino)-*N*-methylpropionamide (ref. 9, MDMP) (track 4) to inhibit protein synthesis by 80S cytoplasmic ribosomes. Cells deflagellated by the *p*H shock method were incubated for 15–45 min after deflagellation in the absence (track 5) or presence of chloramphenicol (track 6). After incubation, a 5-μl aliquot of each sample was taken for hot TCA determination of ^{35}S incorporation into protein[1]. The remainder of each sample was precipitated with 5 ml acetone. For analysis on SDS–polyacrylamide gels, the acetone powder from each sample (which can be stored indefinitely) was dissolved in 2% SDS, 5% mercaptoethanol, 10% glycerol, 60 mM tris (*p*H 7.6), and an aliquot with 2 × 10^6 c.p.m. (except for tracks 3 and 4) electrophoresed on 9% SDS–polyacrylamide gels as described previously[1]. Autoradiographic exposure of the dried gel was for 12 h.

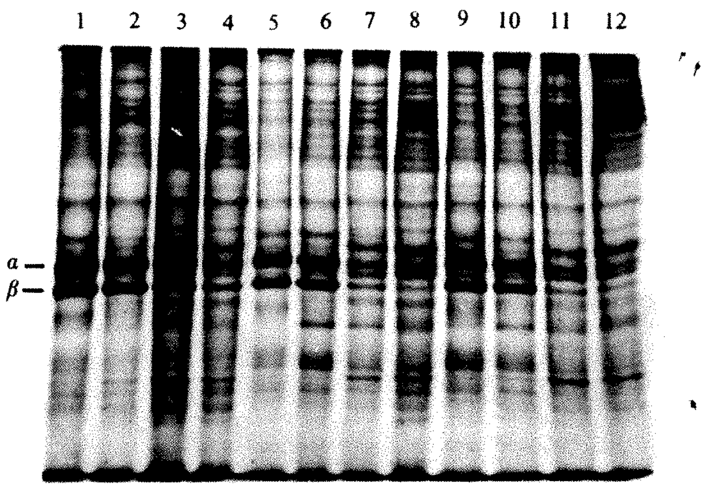

Fig. 3 Tubulin synthesis following deflagellation of control or IBMX-treated cells. Deflagellated cells were labelled for 30-min intervals at 15–45, 45–75, 105–135 and 165–195 min after deflagellation in the absence (tracks 1–4) and presence of 10^{-3}M IBMX given either 15 min before deflagellation (tracks 5–8) or immediately after deflagellation (tracks 9–12). Labelling with $^{35}S-H_2SO_4$ in the presence of chloroamphenicol was as described in Fig. 2.

Thus, the earlier than normal shut off of tubulin synthesis in IBMX-treated cells (Fig. 3, tracks 5–12 compared with 1–4) suggests that a feedback mechanism responsive to increasing tubulin concentrations, could be responsible for the ultimate repression of tubulin synthesis during flagellar regeneration.

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Erratum

In 'Stability of Lotka–Volterra systems' (*Nature*, **264**, 381; 1976) the name of the co-author, J. S. Semura, was omitted.

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matters arising

Geomagnetism and climate

FAIRBRIDGE[1] correlates the Gothenburg geomagnetic excursion (about 13,500 yr BP) with climatic changes associated with the North American glacial record, eustatic changes in sea level, and changes in rainfall and river discharge in the Nile Valley. Such a correlation is interesting considering the possible resulting climatic effects as suggested by Harrison and Prospero[2]. But, Fairbridge in presenting his case ignores the cyclical nature of the continental and oceanic climatic histories. The Holocene, viewed as a whole, is characterised by alternating intervals of glacial advance and retreats. When viewed in this way the correlation of the Gothenburg geomagnetic excusion with a small segment of the climatic history of the earth is hardly justified. Two examples illustrate the danger involved in this kind of correlation. Denton and Karlen[3], from an examination of ^{14}C dates on glacial termini, show that the dates of glacial maxima peak at 200–300, 2,800 and 5,300 yr BP which suggests a recurrence of major glaciers every 2,500 yr. Extrapolating backwards in time, this pattern suggests glacial advances peaking at about 7,800, 10,300, 12,800 and 15,300 yr BP. The Gothenburg excursion corresponds to the time of the Port Huron advance (12,700 to 13,000 yr BP), and/or the Cary/Port Huron interstadial (about 13,300 to 14,000 yr BP), and is no way a unique event when the whole record is viewed. The single correlation of geomagnetic and climatic events at 13,500 yr BP fails to explain similar climatic changes occurring with some regular frequency over the whole Holocene.

In the same way, Fairbridge cites the oxygen isotope record of the Greenland Ice Core in support of his hypothesis. He presents, however, only a small time interval of this record. The isotope record shows a long term climatic trend from 14,000 to 10,000 BP superimposed on which are major oscillations (ref. 4). Given the nature of this climatic record, the correlation of geomagnetic changes and climate at 13,500 yr BP seems purely coincidental.

Finally, it may be suggested that the oceans might provide a reasonable source of material from which a correlation of geomagnetic and climatic changes may be recognised. The oxygen isotope record of foraminifera from deep-sea cores in the Gulf of Mexico, for example, record a major influx of freshwater between 15,000 and 12,000 yr BP[5]. This resulted from the deglaciation of the Laurentide Ice Sheet and subsequent drainage through the Mississippi River Valley. Its correlation in time with the Gothenburg geomagnetic excursion seems pure chance when one examines the ^{18}O record of deep-sea cores throughout the ocean. From such data[6] one is unable to find any major climatic event over the interval of time in question (11–14,000 yr BP).

Thus while the correlation between geomagnetism and climate is interesting, there is no evidence to support it. The main difficulty with the data given by Fairbridge is that it fails to recognise the cyclical nature of the Holocene Climatic record. While many forcing functions may drive it, the case for geomagnetism as a driving mechanism is yet to be made.

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FAIRBRIDGE REPLIES—Hecht insists that I have ignored cycles in climatic history, but this is not so. Three major systems are identifiable: (1) the 200–250-Myr cycle (possibly galactic); (2) the 20,000–100,000-yr Milankovitch cycles (planetary, orbits); and (3) the 11-yr (plus multiples) solar radiation cycle. Each is exogenetic. In contrast, the terrestrial response is greatly dependent on variable palaeogeography—an endogenetic control.

Hecht refers to the excellent glacial chronology of Denton and Karlen. I have made use of it in a paper entitled: 'An 8,000-yr palaeoclimatic record of the 'Double Hale' 45-yr Solar Cycle'[1]. The major coolings on the other hand fall closer to 850-yr intervals, but I do not yet have a convincing casual correlation to offer. The variability does not at present justify Hecht's extrapolations.

In my Fig. 1, covering only the 'small interval' of 8,000 yr, I did not suppress evidence of other oscillations, but merely tried to stress the coincidence of the Gothenburg Event with the largest of them. There still remains a 'chicken-and-egg' problem. The timing of the Gothenburg reversal (closer to 12,500 BP, according to Mörner, personal communications) puts it half-way through the last deglacial phase and thus suggests it was triggered by the massive asymmetric glacial unloading–loading. The latter was catastrophically rapid in geological terms. Thus a general exogenetic global warming trend might trigger an endogenetic geomagnetic phenomenon that in turn introduced a brief cooling. Hecht is right to look at oceanic records, but not for oscillations of only a few centuries. For this information we need carefully analysed cores from lakes where rapid sediment accumulation is taking place.

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Palaeomagnetic stratigraphy of the Koobi Fora Formation, east of Lake Turkana (Lake Rudolf), Kenya

IN their recent article on the magnetostratigraphy of the Koobi Fora Formation of northern Kenya, Hillhouse *et al.*[1] noted that amendments to published correlations of the Koobi Fora stratigraphy were desirable but were unable to take this into account in the evaluation of their results. They noted that alternative dates were now available for the KBS Tuff but did not pursue the inference that if one published ^{40}Ar/^{39}Ar date was questionable, others might be similarly suspect. Some of their statements concerning the geographic location of tuffaceous outcrops were misleading.

An important offshoot from detailed investigation of the evolution of African Plio-Pleistocene Suidae has been the acquisition of correlative information of value both within the Koobi Fora region and between this and other African sites of similar age (J. M. Harris and T. D. White, in preparation, ref. 7). Suid correlative evidence suggests, for example, that the 'Tulu Bor' Tuff of Koobi Fora Area 102 (102/T.IV) is in disagreement with Cerling *et al.*[2], clearly younger than the type Tulu Bor Tuff of Area 129

(129/T.II) or undoubted correlativse (116/T.I, 117/T.III) and is probably of similar age to the type KBS Tuff (105/T.II). A second significant fact to emerge is that the suid fauna from immediately subjacent or superjacent to the Tulu Bor Tuff is evolutionarily equivalent to that from the upper part of Shungura Member B at Omo. A third, equally important, consideration is the apparent absence from the Koobi Fora region of fossiliferous sediments yielding faunas equivalent to those from Shungura Members C–E. It is important also to remember that very few fossiliferous sequences from the Koobi Fora region can be shown to represent other than short time spans and that former lithologic correlations between geographically widely separated areas have to be treated cautiously.

It seems reasonably clear from available vertebrate evidence that the Tulu Bor Tuff is not, in terms of conventional radiometric dates, as old as 3.18 Myr (the date obtained for 116/T.I; ref. 3) and that the normal palaeomagnetic interval observed in that part of the sequence incorporating the Tulu Bor Tuff is probably that separating the Kaena and Mammoth events. Faunal evidence suggests interpretation 2 of Hillhouse *et al.* (Fig. 5 of ref. 1) is strongly to be preferred over interpretation 1. There is no need to postulate secondary magnetisation for the early part of the Area 102 succession (Fig. 4, ref. 1)—the observed polarity being consistent with faunal evidence that this part of the succession is equivalent in age to the normal sequence found immediately below and above the KBS Tuff. The suid faunas from the upper part of the Lower Member of the Koobi Fora Formation are evolutionarily equivalent to those from Omo Shungura Member G and Olduvai Bed I. The conventional K/Ar determination of Curtis *et al.*[4] for the KBS Tuff and other tuffs from the upper part of the Lower Member agree with widely accepted conventional K/Ar dates for Shungura Member G and Olduvai Bed I. It is possible that ^{40}Ar/^{39}Ar step heating methods and fission-track determinations that provide an age of 2.4 Myr for the KBS Tuff (and correlates)[5,6] are better chronometric measures—in which case suid evidence suggests that accepted ages for Shungura Member and Olduvai Bed I are too young by about half a million years. These alternative hypotheses require further research.

References by Hillhouse *et al.*[1] to the 'KBS Tuff' of Area 10 and magnetostratigraphic sections from the Tulu Bor Tuff to the 'KBS Tuff' in Area 131 need correction. Area 10 boundaries were redefined in 1974 and the 'KBS Tuff' referred to by Curtis *et al.*[4] is to be found in Area 12 (12/T.II). The Tulu Bor does not crop out in Area 131. It is present in the closely adjacent Area 129 but here, as at other Koobi Fora localities, faunal evidence indicates an appreciable hiatus (equivalent to Shungura Members C–E) between the Tulu Bor and superjacent (KBS = 129/T.IV) tuff. Moreover, to place data from Area 15 and Area 129 in the same magnetostratigraphic column (Fig. 4, ref. 1) implies firm lithostratigraphic correlation between two areas separated by several kilometres of dense bush cover. It implies also a measure of confidence in this correlation that is not shared by all the participants in the Koobi Fora Research Project.

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HILLHOUSE REPLIES—I thank J. M. Harris for reporting some important new developments concerning the fossil Suidae of the Koobi Fora Formation. These previously unpublished results suggest, as he points out, that a more refined interpretation of the magnetostratigraphy is now possible. As outlined by Harris, the faunal evidence apparently supports interpretation 2 of the magnetostratigraphy presented by Hillhouse *et al.*[1] (Fig. 5), but amendments to interpretation 2 are called for. For example, the normal polarity zone that includes the Tulu Bor Tuff can be correlated with the normal interval (2.92–2.99 Myr) separating the Mammoth and Kaena events, and should not be assigned to the end of the Gauss normal epoch (2.41–2.84 Myr) as in interpretation 2. Also, the absence from the Koobi Fora region of fossil faunas equivalent to those from Shungura Members C–E[2] suggests that sediments representing the upper half of the Gauss normal epoch and the lower part of the Matuyama reversed epoch are not present in the Koobi Fora Formation. The adoption of this amended interpretation depends on the strength of the evidence from the fossil Suidae weighed against radiometric dating and palaeontological data from a diverse selection of fauna. We eagerly await publication of Harris's results so the matter can be openly evaluated by geologists and palaeontologists.

Harris is correct in pointing out that because of the 1974 redefinition of area boundaries the 'KBS Tuff' referred to by Curtis *et al.*[3] and by us is now in area 12. As Harris points out, the Tulu Bor does not crop out in area 131. In our Fig. 3 of ref. 1 we emphasised this by not showing the tuff in the stratigraphic column and by stating in the caption, 'The Tulu Bor Tuff is believed to be located approximately 6 m below the base of this section.' This statement was based on Fig. 2 of Leakey[4], which infers the location of the Tulu Bor in area 131. Concerning the observation that the results from areas 15 and 129 are not from the same magnetostratigraphic column, Harris may have missed our comment, " 'Area 129' column is a composite: the upper portion was from area 129, the lower from the adjoining area 15." Finally, we note that Harris's comment that there is no need to postulate secondary magnetisation for the early part of the area 102 succession is in accord with the following statement in our article: "Identities of tuffs are unknown in the lower portion of area 102 column, so we can no longer assume that this section was overprinted with normal polarity."

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[1] Hillhouse, J. W., Ndombi, J. W. M., Cox, A. & Brock, A. *Nature* **265**, 411–415 (1977).
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Maya dates AD 352-1296

PLANETARY conjunctions, in my recent article[1], were used to fix the floating chronology of the Classic Maya codices and stelae. Many planetary configurations, however, repeat themselves after 59.1 yr so that multiple solutions are possible. I first supposed as a limiting condition that the conventional (Goodman–Martinez–Thompson) dates were in error by not more than 30,000 d, so postponing investigation of a possible solution 1,541 days beyond that limit. I found that an adjustment by 27.3 yr brought the planetary cycle into phase, and the adjacent solution 59.1 yr or 21,574 d greater was not fully investigated. Either solution would fit the conjunctions of AD 650/3, 709/11 and 770. My present work[2], has shown that it was my first solution that was spurious: 615,824 d must now be added to all Mayan Day Numbers to get Julian dates, and 59 yr 24 ± 1 d must be added to the Maya dates in my previous[1, 3] articles. This is 86.4 (86 yr 4 months and about 3 d) yr or 31,541 d greater than the conventional dates.

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[1] Schove, D. J. *Nature* **261**, 471–473 (1976).
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reviews

Science as a never-ending quest

J. R. Ravetz

The Nature of Science: The History of Science in Western Culture Since 1600. By David Knight. Pp. 215. (André Deutsch: London, 1977.) £4.95.

In comparison with many other disciplines studying human behaviour, the history of science is quite peaceful and self-confident. Although divergences of approach have always existed, they have never polarised the whole field. The present book represents a considerable shift in the consensus of what the history of science is all about, and yet it is done in a relaxed, matter-of-fact way, totally devoid of programmatic pronouncements.

What David Knight argues, in *The Nature of Science*, is that it lies as much in the activity of research and its context, as in the results we accept as valid. The supposed 'hard core' of objective facts, which could be studied on their own by philosophers and historians, is now blended into the matrix —social and intellectual—of scientific work.

In order to exhibit this view of science, David Knight must approach history in an unorthodox way. Instead of treating 'periods' in chronological sequence, he uses 'themes'. We have a progression, from the more philosophical ("Science as a Complex Activity", and ". . . as Explanation"), to the more social, linking science to its community, and discussing careers, Government and utility. The bridge-section is on "Science and Not Science".

It is very refreshing for a person familiar with the history of science to see the materials sorted in this way. But this arrangement has its costs, most notably in the loss of a chronological sense for the whole story.

The general literary style is relaxed and conversational. The book makes enjoyable reading, but the shape of events in history becomes quite blurred as the narrative slides from one topic to another.

The history that is told in such a way, especially in a small book covering such a wide range, inevitably becomes a personal statement. The materials reflect the author's own interests; here, social and institutional aspects of English science absorb much space. Also, the text is liberally sprinkled with the author's own generalisations and morals. They are recognisable as such, and fit with Dr Knight's commitment to the view that history, like natural science, is a never-ending quest, best not left exclusively with professionals.

Everyone will find fault or favour with his/her own selection of wisdom. I am intrigued by the suggestion that eighteenth-century chemistry was hindered by the Newtonian orthodoxy; this is a far cry from the classic ridicule of 'phlogiston' as the nonsense theory that awaited overthrow by Lavoisier. Some throwaway statements have rather strong implications; to say that conservatives were "naturally enough often right in resisting innovations", opens up new and challenging perspectives on the history of scientific revolutions and indeed on the methodology of science.

So here is a book to be enjoyed rather than dissected; a very useful introduction to a growing new historiography of science. ☐

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Genetic expression in plants

Molecular Aspects of Gene Expression in Plants. Edited by J. A. Bryant, Pp. 338. (Academic: London, New York and San Francisco, 1977.) £9.60; $21.

This seven-chapter volume has been compiled by four authors, one of whom is the editor. Together they have many years experience with plant nucleic acids, and they have pooled their joint approaches and expertise to produce an overall view in what the dust cover calls "the first book to deal specifically with gene expression in plants".

The first half is a mine of basic information on the properties and functions of DNA, RNA and proteins in cells, and freely uses information from non-plant sources. The first chapter on nuclear DNA deals effectively with the extraction and properties of DNA, the structure of chromosomes and the modifications and variations that can occur in the genome following synthesis and turnover of DNA *in vivo*. Bryant has done a service in confining the term "nucleohistone" for the DNA–histone complex and "chromatin" for the DNA–histone–acidic protein complex throughout the volume. Although the classification given for histones is the "f" nomenclature, it would have been helpful to undergraduate readers if the "H" nomenclature, which they will surely encounter in the literature, had been included. Nucleosomes receive brief mention only, and although buoyant density fractionations of DNA are discussed, the use of sucrose density fractionations is not.

The chapter on RNA structure and metabolism provides a lucid account of the different classes of RNA, outlining their separation and function as intermediates for the translation of genetic information in protein synthesis. Considerable space is given to the topic of precursor RNA molecules and their processing to ribosomal RNA in both prokaryotic and eukaryotic plants. The possible role of tRNA molecules and the stability and function of mRNA are discussed in relation to well studied examples of the control of transcriptional and translational events in plants.

There follows a straightforward account of the translation steps in protein synthesis with frequent comparisons with bacterial and mammalian systems. Most of the plant information has been gleaned from the extensive work on germinating seeds and protein-synthesising cell-free systems from wheat-germ. Attention has properly been drawn to the gaps in our knowledge for plants. The diagrams and schemes are somewhat repetitious, but the points are very clearly made.

A useful chapter is devoted to gene expression in chloroplasts and mitochondria, to include the basis of their role in cytoplasmic inheritance and the degree of their developmental autonomy. The nature and replication of chloroplast DNA, the transcription of RNA and the assembly of chloroplast proteins (particularly fraction I protein), are presented in an interesting way. The section dealing with mitochondria is brief, but sets out present knowledge of their DNA structure and RNA transcription products, together with the meagre information on their protein synthesis.

The factors linking DNA replication with cell division are treated at length in a wide-ranging and well presented chapter on the cell cycle. Attention is focussed on patterns of nuclear RNA and cytoplasmic protein synthesis and the modifications in chromatin that occur through the different phases of the cycle. Changes in enzyme activities that accompany these cyclic events are well presented. Attempts to isolate control factors in mitosis in plants are described and the role of cyclic AMP, though mentioned, is properly treated with caution. The overall information is neatly summarised and the reader will appreciate the comprehensive diagrammatic presentation of the known regulatory events occurring through the cell cycle of many eukaryotic and prokaryotic types, together with the leads into hormonal and nutrient gradients that can turn on and turn off division in plant cells. Although to some extent the conditions that trip the switches are known, the reader will learn that the operating mechanisms for the switching systems are little understood.

The chapter dealing with molecular aspects of differentiation rests uneasily within this volume, for although it describes in detail the ultrastructural morphogenesis of certain cell types it is not concerned directly with the mechanism of gene expression. It includes, however, a useful and well reasoned survey of transcriptional, translational and post-translational control in protein synthesis.

In the final chapter on plant growth substances we learn that the term "plant hormone" is going out of usage (which I doubt) and that these regulatory substances are the keys to our understanding of differentiation and organisation (with which I concur). Two examples of altered gene expression are considered in detail. One, the control by gibberellic and abscisic acids of the synthesis and secretion of hydrolytic enzymes (especially α-amylase) in barley aleurone cells, and secondly, the role of auxin in the control of cellulases during cell expansion. These aspects of differentiation are well integrated with the earlier chapters on the involvement of nucleic acids and proteins, and include discussion of the conclusions that may be drawn from hormone-modified changes in incorporation and enzymic activity studies.

Taken as a whole, the content of the book is stimulating and very readable. The bibliography and reference system are excellent, and the illustrations and tables have been carefully selected to be simple and instructive. This book is timely and relevant, and will be much used by those seeking information on genetic expression in plants. ☐

Daphne J. Osborne

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Peasants to tractor drivers

Cognitive Development: Its Cultural and Social Foundations. By A. R. Luria. Pp. xvi+175. (Harvard University: Cambridge, Massachusetts, 1976.) £9.40.

THIS book reports a pioneering study done in the early 1930s by the gifted Russian psychologist, Luria, to determine the impact of collectivisation, literacy, and newly introduced technology on the peasants of remote Uzbekistan and Kirghizia. It looks for the impact on the cognitive and intellectual processes of those affected: their perception, categorising, reasoning, and self-image, in the conviction that these processes are not simply biologically given but are shaped by cultural–historical processes—a view now widely shared by Marxist and Western psychologists alike, though by no means so in the 1930s. Interestingly enough, the work did not see the light of day in book form in Russia until 1974, its publication having been 'discouraged' as disrespectful of minority group sensibilities when the results were first reported at a time when official Soviet policy was strongly directed toward the integration of minority nationalities, shades of the American experience in the late 1960s with respect to the Blacks in the United States.

The point of view of the book is 'developmental' in the sense that the experience of schooling and modern technological 'collective' life is seen as 'completing' the stages of mental development of illiterate peasants, whatever the age at which they were exposed to the new influences. Although the methods used were 'clinical–experimental' procedures, involving various kinds of sorting and reasoning tests followed up by probing interviews to get at the basic reasons for subjects operating as they did, the results are quite clear at the factual level. Illiterate peasants tend to be much more concrete, function bound, 'complexive' (in the sense of thinking thematically rather than syllogistically), and tied to immediate experience than are their comperes who had been exposed to schooling and the cooperative–technical ways of the collective farm. Luria characterises the advance as 'the leap from the sensory to the rational.' Even with respect to characterising themselves, the illiterate peasants have great difficulty achieving detachment, seeming quite unable to characterise personal traits in a fashion free from functions and duties: they are more situation bound than their literate brothers.

The issue that the book poses is one of interpretation. Granted that 'cultural–historical' factors are important, is it a question of different intellectual powers being available to the two groups on the basis of experience, or is it rather the ways in which human beings, using the same underlying powers, adapt intellectually to their circumstances? An example from the test of syllogistic reasoning illustrates:

LURIA: Cotton can't grow where it's cold, and it's cold in England. Does cotton grow there or not?

KHAMRAK (an illiterate miller from a remote village): If it's cold it won't grow, while if it's hot it will. From your words, I would have to say that cotton shouldn't grow there. But I would have to know what spring is like there, what kinds of nights they have.

Khamrak, to be sure, is distrustful of reasoning from *a priori* premises. But may it not reflect a deeper adaptation to a mode of life where skill and experience count more than in a technical society, such that knowing *that* is subordinated to knowing *how*?

The work of Cole and Scribner, of Horton, and of Labov, all indicate that cultural factors produce a different deployment of universal, basic cognitive and intellectual processes of different peoples, that these deployments do not on close inspection turn out to be any less 'rational' when applied to one set of circumstances than any other. Personally, I would rather trust Khamrak with local agricultural decisions than most of my Oxford undergraduates who could solve the cotton syllogism in a trice. Perhaps the fault lies with the use of a developmental model to account for differences produced by differingly technical cultures.

Be that as it may, the book is rich in data and observation on the nature and transformation of the peasant mentality into the more familiar 'rational' reckoning of those who went on to run the tractor pools and marketing arrangement of the *kolkhoz* that transformed Soviet agriculture and life in the country. Luria brings a rare genius to the task, managing, as he has in his past books, to find the germ of generality in the everyday particulars to which he brings his observational skills.

Jerome Bruner

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Biomolecular mixture

Intermolecular Interactions and Biomolecular Organisation. By A. J. Hopfinger. Pp. xviii + 395. (Wiley Inter-Science: New York and London, 1977.) $33; £19.50.

NEW results in science are published and then reviewed in journals specialising in broad or narrow topics of current interest. Books should be different in that they represent a new synthesis of fact and theory collected and distilled into a coherent and useful entity. Unfortunately, we are facing a deluge of books which offer very little that has not earlier been given in closely related form. The volume under review, although attractively titled, is a compilation of disjointed topics with no visible thread leading from one to the other. One cannot hope to learn from it about the theory of solutions of macromolecules, or conformational analysis by nuclear magnetic resonance, or quantum mechanical calculations, nor can one form a critical opinion about how these connect to the problem of drug design, for instance, to which a whole chapter is devoted. I found particularly revealing the admission of the author that large portions of his book are indeed taken (p145 or p252, for instance) "from the excellent review" of other authors!

After trying to examine the extensive subject matter somewhat more closely, I am reminded of a story told by the late author, Maurice Samuel, in his quest to evaluate the widely ranging work of a famous contemporary historian. All fellow historians approached indeed acquiesced that his study of history seemed highly significant; however, as far as their own speciality was concerned, they voiced grave reservations. Trying to evaluate not only the breadth of the present work, but rather the depth to which individual topics are treated, particularly those with which I am familiar, I ran into similar difficulties. To mention a few examples only, our own work on hydration of DNA is incorrectly quoted (p160), lumped together with other quotes in purely irrelevant fashion. Concerning our work quoted in the footnote of Table 8-1, viscometry of DNA cannot be used directly to compute the number of intercalated dye molecules. On p265, part of the enormous Table 12—1, the author reprints an incorrect finding first made in 1958, and then often repeated, that glutamic dehydrogenase is a zinc enzyme with a molecular weight of 1,000,000 containing two to six Zn molecules. For many years now it has been known that glutamic dehydrogenase has a molecular weight of six times 56,000 and does not contain Zn. The discussion on nucleohistone aggregation and chromatin structure in chapter 13 is largely outdated and would be unintelligible to the uninitiated. Space does not permit me to go into other examples, which abound.

The proof-reading is sloppy beyond the border of insult. Having myself recently published a book, I know how difficult it is to win the battle with publisher and printer. Yet one must try. The author is responsible that the same name is spelt Fahien, Fehiem, Fahiem on one page(152), Subivana and Subirama (the correct name is Subirana) on p204, and Schleich, Scheich and Schiech in chapter 12. There are many more such examples. A carefully proof-read name index would have been helpful in a work which draws on so many varied sources.

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Science policy yearbook

Science and Government Report International Almanac, 1977. Edited by Daniel S. Greenberg. Pp. 305. (Science and Government Report: Washington DC, 1977.) $54 before 1 September; afterwards $75.

THIS first volume in what is intended as an annual series introduces the idea of a 'science policy yearbook'. Part I consists of 20 chapters which survey, nation-by-nation, major recent developments in science and technology policy. Most authors are experienced science journalists (not a few of whose names will be well known to *Nature* readers), and the chapters are concise, informative and wholly unpolemical. Each covers more or less similar ground: changing priorities; research and development expenditure; changes in government research organisation and science policy advisory machinery; and issues which in the country concerned have been publicly contentious.

I personally enjoyed this part of the book and can recommend it to anyone who wants to keep broadly in touch with what is happening on the interface between science and government in a wide range of countries. Though more readable than the products of international organisations active in this field, it lacks the systematic quantitative data which often makes their reports a useful basis for further analysis.

Part 2 of the book, an international directory of major scientific organisations and science policy personnel, though commendably up to date, is curiously haphazard in its coverage. Thus, although we find listed British Departmental Chief Scientists and Britain's Scientific Counsellors overseas, the only organisations whose addresses and functions are given are the Central Policy Review Staff in the Cabinet Office, the SRC, MRC and Department of Energy.

Part 3 gives excerpts from eight recent (mostly American) science policy documents, including President Carter's campaign speeches and the NIH guidelines on recombinant DNA experiments.

On balance, the yearbook idea is an interesting one, and libraries might wish to acquire this first effort notably for the contents of Part 1.

Stuart S. Blume

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will
change
your
reading
habits

Early development and gene activity

Gene Activity in Early Development. Second edition. By Eric H. Davidson. Pp. xvi+452. (Academic: London and New York, 1977.) £11.30.

THIS is an enlarged and completely rewritten edition of Dr Davidson's successful 1968 book of the same title. It is the only comprehensive review, at the molecular level, of early animal development, and provides a scholarly and unbiased account of the field as it now stands. It will be invaluable to research scientists and advanced students interested in this field.

The book consists of eight chapters, covering the following topics; the constancy of the genome; the initiation of genomic control and of cell differences; the changing populations of proteins and RNAs in embryos; cytoplasmic determinants, and oogenesis. Each chapter is preceded by a summary of the principal facts. Within most chapters, results are presented according to taxonomic groups. This is a wise decision, because there are remarkable differences in the morphological and molecular characteristics of development of different animals, a situation which has made it impossible so far to identify any universal mechanism of development applicable to all animals. One chapter provides a very helpful explanation of renaturation and molecular hybridisation experiments, such as have been used in much of the author's own work. One of the particularly good characteristics of the first edition which has been retained is the survey of classical embryological literature. This is especially valuable since most of those engaged in the molecular analysis of early development have no familiarity with, or convenient access to, early literature of this type. A detailed and very representative bibliography of original papers and their titles, up to the middle of 1976, is provided.

The general theme which lies behind most of the work reviewed in this book is the importance of gene activity in development. The author's view is that during oogenesis (egg formation) gene products are accumulated. Soon after fertilisation, they are moved to different positions in the egg, and as a result, they (or other molecules formed by their activity) cause activation of different genes in different cells. These activated genes start to make their own products during gastrulation and at later stages. This represents a fairly conventional view of early development, having been favoured by many writers since the end of the last century. Certainly the detailed evidence summarised in this book does a lot to bolster one's confidence in this hypothesis.

It is currently fashionable to devote much attention to cell surface molecules, and those who fall into this class may feel that this aspect of the molecular repertoire of cells is lightly represented. There is no evidence at present, however, that any of these molecules are causally connected with development. An area of relevant information which is not covered in this book concerns the molecular differences between highly specialised adult cells. It is possible that a detailed molecular description of differences between adult cells may lead more directly to an understanding of early developmental processes than a description of molecular changes which take place in embryos themselves.

As is evident from reading this book, two factors restrict progress in this field. One is the lack of a detailed description of the activity of single known genes; the other is the absence of a way of distinguishing genes and gene products which are important in development from those which merely accompany this process in a non-causal way. Procedures are becoming available by which these kinds of information can be obtained; but for many years to come, this book is likely to be a standard reference work for all those interested in early development and gene activity. **J. B. Gurdon**

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Tilting at windmills

Ecological Sanity. By George Claus and Karen Bolander. Pp. xv+592. (David McKay: New York, 1977.) $16.95.

IT is difficult to understand why the David McKay Company of New York have bothered to publish this book in 1977. They very honestly admit in a note at the front that the research (*sic*) on which it was based was completed in 1972, and that it was delayed by "production difficulties beyond our control or that of the authors". The authors' Preface has a dateline "Vienna 1972". The publishers state that they are "confident that the continued significance of this book will be apparent to the reader". Unfortunately this does not apply to this reviewer.

Ecological Sanity sets out to give a critical examination of the statements made by the so-called "Ecological Lobby", particularly in the United States. The lobby is accused of putting forward statements which cannot be supported by sound research, and for making premature Doomsday announcements. This is all set out at great length, for the book is not far short of 300,000 words. Had it appeared in 1972 it might have been of some interest, although even by then many other authors had already produced more effective criticisms of the errors put forward by the more extreme environmentalists who had assumed the title of "the ecologists". It is true that some of these extremists are still unrepentant, although many have either recanted or disappeared from the scene. Claus and Bolander are, for the most part, tilting at windmills, for the situation is very different from that which obtained five years ago. A far greater number of people, particularly those in authority, are able to view the scene more rationally. Thus, the World Health Organisation still recommends DDT for malaria control in appropriate situations, although the dangers of misuse are fully recognised. The more absurd statements of the lobby have been recognised as such; their other efforts to keep the rest of us on our toes, is probably a good thing.

There has, of course, been controversy about pesticides, particularly the organochlorine insecticides. The 275 pages of small print (almost half the book) on this subject is particularly unsatisfactory. It is true that, in the late 1960s and early 1970s, some environmentalists extrapolated unjustifiably from the results of research. There has, however, been a great deal more work done in the past five years. Some of this disproves conclusions based on extrapolations, some supports conclusions hitherto unproven. This omission of what is now the major part of the relevant research means that the book is of interest only to the historian of the bye-ways of science.

Yet there is still a need for us to be reminded of the need for ecological sanity, even if this book does little to achieve this objective. Environmental dangers still exist, and it is as bad to exaggerate their importance as to refuse to recognise their existence. Resources will always be limited, and environmental protection will always be subject to compromise. This is unfortunately not as glamorous as extremism from the "right" or from the "left". **Kenneth Mellanby**

Kenneth Mellanby is Editor of Environmental Pollution, *and was formerly Director of Monks Wood Experimental Station, Huntingdon, UK.*

obituary

Sir Graham Sutton

Sir Graham Sutton, former Director-General of the Meteorological Office, who died on 26 May 1977 at the age of 74, achieved eminence early in his career by fundamental contributions to the theory of atmospheric turbulence and diffusion.

His work in this field began in the late 1920s, when atmospheric boundary-layer theory was at a primitive stage. It had, however, become evident, from early observations of diffusion and from pioneering theoretical studies by G. I. Taylor and L. F. Richardson, that the diffusion of a plume of smoke could not be explained on the basis of a constant eddy diffusivity analogous to, but very much larger than, the molecular diffusivity of kinetic theory. The starting point of Sutton's theory of turbulent transfer was the combination of Taylor's famous theorem, for the kinetics of passive particles carried in a turbulent fluid, with the classical Prandtl mixing-length theory. This led to an explicit height-dependent form of the eddy diffusivity. It also led to the result that the spread of a plume of gas or fine particles issuing from a point in a wind was proportional to distance raised to a power between $\frac{1}{2}$ and 1, the precise magnitude of this exponent and of the proportionality constant being determined by the form of the increase of wind with height above ground.

The consequences of this work have been wide-ranging and long-lasting. It provided the basis for the first formulae representing the effect of meteorological conditions on the concentration-field downwind of a source of gas or particles, and Sutton's point-source formulae, at first in restricted use in chemical warfare studies, became widely known immediately after the 1939–45 war in the context of industrial air pollution. It also was a stimulus for continuing and far-reaching boundary-layer studies, notably of the wind profile, the aerodynamic drag of the earth's surface and the factors determining evaporation from water surfaces and transpiration from vegetation.

Oliver Graham Sutton was born in Cwmcarn, Monmouthshire, in 1903. He took his degrees in mathematics at Aberystwyth and Oxford and his first appointment was to a lectureship at Aberystwyth. Two years later, in 1928, he was persuaded by the late Sir David Brunt to join the Meteorological Office and shortly afterwards he was seconded to the War Office establishment at Porton, where the aforementioned work was carried out.

Clair-Continental

In 1942, with his potential for scientific administration already strongly evident, he was required to leave his meteorological team for wider wartime responsibilities as Superintendent of Research at Porton, and he was drawn into further fields of administration in posts as Superintendent of the Tank Armament Research Station (1943–45) and Chief Superintendent of the Radio Research and Development Establishment (1945–47).

In 1947 he returned to individual scientific work in the position of Bashforth Professor of Mathematical Physics at the Royal Military College of Science, Shrivenham, though here also wider responsibilities were to be placed on him, as Scientific Adviser to the Army Council (1951) and Dean of the Royal Military College (1952–53). His individual scientific achievements were recognised in his election as a Fellow of the Royal Society in 1949.

During this phase of his career he was able to turn to more general writing on the fundamental science of boundary-layer meteorology, an ambition on which he had made a start while at Porton. His first published effort was *Atmospheric Turbulence* in the Methuen Monograph series (1949) and this was followed by his well-known major work *Micrometeorology* (1953), in which he covered the most important topics in the physics of the surface air layers. Although much had already been written on the qualitative aspects of those topics, *Micrometeorology* was the first connected account of the relevant principles and crucial observations in a full mathematical–physical setting. His capacity for exact mathematical analysis and lucid exposition of complex physical interpretations came out here to the full, and he was to make good use of the latter gift over more than two decades of writing for less specialised readers, as for example in his *Science of Flight* (1950) and *The Weather* (1974).

In 1953 Dr Sutton accepted an invitation to become Director-General of the Meteorological Office, and for the next twelve years devoted his mature powers of leadership, scientific judgement and administrative skill to the formidable task of guiding its developing activities. His period of office saw the emergence of a fully-developed research division with the scope and reputation of a leading institute of meteorology. Technical resources were greatly expanded and included the installation of high-speed computing facilities. The services which meteorological science could usefully offer were extended far beyond those originally designed for weather forecasting and aviation. At the same time the hitherto scattered main branches of the Office were brought together in the present unified headquarters at Bracknell.

Sir Graham (he was knighted in 1955) planned to retire from professional activities in 1965, but he was then persuaded to be first chairman of the Natural Environment Research Council. Here again his outstanding abilities as an administrator and his qualities of leadership were turned to an urgent and important task, that of ensuring the rapid and successful development of the new organisation for promoting the various sciences of the natural environment.

The many recognitions and awards received by Sir Graham included the Symons Memorial Gold Medal of the Royal Meteorological Society (1959), the International Meteorological Organization Prize (1968) and the Frank A. Chambers Award of the United States Air Pollution Control Association (1968). There were also countless demands on his time for work on committees and in other organisations, and to many spells of service as chairman he invariably brought a balanced judgement, a diplomatic touch and a quiet sense of humour. While Director-General of the Meteorological Office he took a prominent part in the activities of the World Meteorological Organisation, especially as a member of its Executive Committee. He was President of the Royal Meteorological Society 1953–55. He also found time for the duties of a Justice of the Peace and for those of a member of the Council of the University College of Wales, Aberystwyth, of which he was Vice-President in his last few years.

F. Pasquill

A. D. Walsh

PROFESSOR A. DONALD WALSH FRS died in Dundee, after a long illness, on 23 April 1977 in his sixty-first year. He was educated at Loughborough Grammar School and Corpus Christi College, Cambridge, where he was a Mawson Scholar. In 1938 he started research in vacuum ultra-violet spectroscopy under the direction of Professor W. C. Price and continued as an ICI Research Fellow until 1949, when he accepted a lectureship at Leeds University. In 1953 he was promoted to a Readership, and two years later was invited to take the Baxter Chair of Chemistry at Queen's College, Dundee, which was then part of the University of St. Andrews. In 1967, when Queen's College became the University of Dundee, Professor Walsh was appointed Dean of the Science Faculty. He was elected to the Royal Society of Edinburgh in 1959 and to the Royal Society in 1964.

Donald Walsh will always be remembered for his enthusiasm. Whether he was lecturing to a first-year class, discussing his latest research, or watching the flight of a bird while driving erratically along a country road, he radiated a fascinating and infectious enthusiasm for his subject.

It was this enthusiasm which marked his rise to the heights of his profession. The investigation of the electronic structure of small molecules and the nature of valence and chemical bonding were, for him, an absorbing study throughout his career. The topic suited his enquiring mind ideally, for he loved to put his ideas to the test and spent very many happy days fitting together his theories and his experimental spectra.

During the war, when it became necessary for research to have more practical application, Donald Walsh became involved in problems of gaseous combustion, such as the suppression of the exhaust flames which revealed the presence of our aeroplanes in the sky at night. After the war, he became more interested in the mechanism of gaseous combustion reactions, and in particular with the mechanism of anti-knocks in petrol engines. In this he was associated with Riccardo Ltd and later with Octel Ltd who still employ a number of his former students. Those privileged to hear him present his research papers in the 1950s and 1960s will clearly recall the rival theories on anti-knock action put forward by Professors R. G. W. Norrish and A. D. Walsh, the debates being enlivened by the completely different personalities of these two eminent scientists. Donald Walsh was fond of simple experiments to demonstrate his ideas. One remembers the hydrogen peroxide which usually hit the ceiling in a demonstration that peroxides are rapidly decomposed by particulate lead oxide (an important part of his theory of the anti-knock action of lead alkyls.)

In Dundee his gentlemanly charm and hospitality quickly gained for him the loyalty of a department which rapidly grew under his direction and enthusiasm. His research students enjoyed many happy Saturday afternoons helping him construct his large garden, and they appreciated the generous quantities of Elin Walsh's magnificent teas which contrasted so markedly with student fare.

When Dundee gained independent status as a university in 1967, Donald Walsh was the obvious choice for Dean of the Faculty of Science—a Faculty which might have disappeared earlier but for his efforts. Extra duties did not deter him, and he habitually arrived in the Department at 6 a.m. after an overnight journey from London—he was still there at 7 p.m.

He bore his long fight with Parkinson's disease with typical fortitude as he was gradually forced to retire from chemistry and from the department to which he had devoted so much of his time and energy.

D. E. Hoare
P. A. Warsop

R. D. Brauer

RICHARD DAGOBERT BRAUER, who was one of the leading pure mathematicians of this century, died on 17 April 1977 at Belmont, Massachusetts. Born in Berlin in 1901, he studied at the university there under the distinguished algebraist Issai Schur. He taught at Königsberg until the political oppression in Germany forced him to leave in 1933. He held temporary posts at the University of Kentucky and the Institute for Advanced Study in Princeton. In 1935 he went to the University of Toronto, where he stayed until 1948; he then returned to the United States, and held chairs at the Universities of Michigan (1948–1952) and Harvard (1952–1971).

Brauer's work on the representation theory of groups has been a major influence in the development of algebra during the past half-century. Some of his earlier papers were concerned with the representations of semisimple continuous groups—Hermann Weyl's fundamental work on this subject appeared at about the time (1925–1926) when Brauer was presenting his doctoral thesis. Brauer's methods were more algebraic, and in some ways simpler than Weyl's. In fact Brauer worked as assistant to Weyl in Princeton in the year 1934–1935, and they wrote there a joint paper on spinors, which is the classical exposition of the mathematical background to Dirac's theory of the spinning electron.

From 1935 onwards, Brauer was increasingly concerned with the representation theory of finite groups. He developed a beautiful and deep theory of modular characters, which gave a completely new insight into the study of group characters. Group characters had been introduced in 1896 by Frobenius, and had already given, in the first decade of this century, some remarkable results on the structure of finite groups. It was Brauer who, thirty years later, resumed this work, and by the end of the 1950s had laid the foundation of a method for attacking the very difficult problem of the classification of finite simple groups. His pioneering work was one of the starting points of a great advance in this problem. Brauer's active involvement in this research continued right up to the end of his life.

During his long career Richard Brauer received many academic honours and held some of the highest positions in his profession. But he retained a very gentle and unassuming manner, and a concern for the problems and interests of other mathematicians. His modesty about his own great achievements was proverbial—a mathematical conversation with him would usually become a conversation about the other person's work. A first impression of unworldliness was deceptive; he used his time very efficiently, and achieved a great deal in an unhurried manner. He became a kind of elder statesman among American algebraists, and his advice was sought and valued for its wisdom and humanity.

He is survived by his wife Ilse (née Karger) whom he married in 1925; also by his brother Alfred, and his two sons George and Fred, who are all mathematicians.

J. A. Green

what's new–chromatographs

In addition to the regular Newly on the Market section in *Nature*, special features on a particular type of laboratory equipment or instrumentation will be published in this and forthcoming issues. The issue of 20 October will be on Spectrometers and ancillary equipment.

The purpose of a special feature is not to give listings of every item in the range of the manufacturers mentioned but to take a variety of equipment of interest in that feature area and give an idea of what kinds of instruments are available and from whom. There is a Reader Enquiry Coupon facing the inside back cover.

EDT Research now have a demonstration Jobin-Yvon Chromatospac preparative liquid chromatograph in the UK. This instrument has a loading capacity of 100 g of sample per injection and can provide high performance (typically 7,000 theoretical plates per m with TLC grade silica). Many different types of stationary phase have been used successfully including alumina and silanised silica for reverse phase. A unique pressure packing system for the stationary phase provides excellent reproducibility. The instrument also has the capacity to operate in the partial elution mode, as the stationary phase can be completly removed from the cylinder.

Circle No. 37 on Reader Enquiry Coupon

Phase Separations Ltd present HPLC adsorbents. Spherisorb is a range of highly efficient, totally porous, particles for HPLC and the range of materials is based upon 5 μm, 10 μm and 20 μm particles of alumina and silica with a variety of chemically bonded phases for reverse phase chromatography. The Model 1203 from Laboratory Data Control is a very sensitive ultraviolet detector designed specifically for use in HPLC and has selectable ranges from 0.002 to 2.048 aufs. The new detector has a noise level (typical short term) of 2×10^{-5} au (P to P). A new range of glass capillary columns is now available. Both wall coated open tubular and support coated open tubular are available with a wide variety of stationary phases.

Circle No. 38 on Reader Enquiry Coupon

Knauer present a range of equipment: a differential refractometer for analytical and preparative use; a high temperature refractometer up to 160 °C; ultraviolet filter photometer (254 nm); a spectral photometer (200–600 nm); uv/ri dual detector; a double filter photometer (254–600 nm); a fluorescence detector; combination ultraviolet and fluorescence detector and conductivity detector. Knauer columns have specific advantages, namely, the inside diameter of the stainless steel column is especially honed to provide a highly polished surface and precise concentricity; symmetrical construction makes 'backflush' possible; the column ends have integral machined threaded terminations for safety. A replaceable seal is used instead of a ferrule, so columns can always be refilled. Stainless steel sieves and a fibreglass filter are used to retain all particules larger than 2 μm. Knauer HPLC columns are modular in construction. The optimum separations obtained with analytical columns can also be expected with the preparative columns. The preparative columns (16 mm i.d., length 25 cm and 50 cm) may be packed with the same finely granulated packing materials as the analytical columns (4, 6 mm i.d.). The use of a 'stream distributor' in the column, produced a uniform spread of the sample onto the surface of the column and low dead-volume elute flow.

Circle No. 39 on Reader Enquiry Coupon

Knauer Dualdetektor

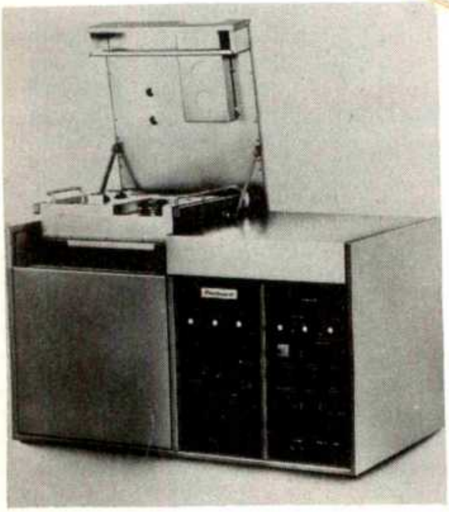
Rofin announce the Chromatix KMX-6, a revolutionary low angle light scattering photometer which utilises the spatial characteristics of a small, single transverse mode laser. The patented design allows measurement of scattering at forward angles of less than 2° from the incident beam, and permits weight-average molecular weights to be obtained on an absolute basis in minutes, without recourse to an arbitrary standard. At such low angles, weight-average molecular weights may be calculated from scattering measurements at a single concentration and angle, if the solute second virial coefficient is known. When the second virial coefficient has not been determined, the data need only be extrapolated to zero concentration by making measurements at two or more concentrations: no angular extrapolation is required. Rapid instrumental response makes high speed kinetic measurements possible, and when combined with a computer and gel permeation chromatograph, molecular weight distributions can be determined. The high sensitivity and many unique design features of the KMX-6 permit it to be applied to a variety of applications in many different scientific fields, such as molecular weight determinations, molecular weight distribution (with GPC), macromolecular kinetics (enzyme and antibody-antigen reactions, dimerisation, etc.), single particle fluorescence and nephelometry, molecular interactions (virial coefficients), molecular conformation (radius of gyration), molecular anisotropy (depolarisation), Rayleigh spectroscopy (diffusion coefficients), Brillouin spectroscopy (acoustic modulation), and refractometry.

Circle No. 40 on Reader Enquiry Coupon

Whatman supply a vast range of chromatography columns and packings and modern liquid chromatography of nucleic acid constituents and other other naturally occurring compounds. Whatman offers the widest choice of prepacked columns and loose media commercially available. Silica gels, aluminas, polyamide, bonded phases for reversed-phase and normal phase chromatography are available in both prepacked, pretested columns and as loose media. Many of these have direct application in HPLC for separation of different classes of biologically significant compounds, e.g., steroids may be separated on Partisil. Various types of Partisil appropriate for different types of chromatology are available e.g., Partisil-10 PAC, for normal phase partition, HP2C, a complement to Partisil-10 ODS, a microparticle non-partition bonded phase for

reverse-phase HPLC and so on. Both pellicular and microparticulate media are available. The microparticle-support bonded ion exchangers are increasingly important ion exchange media because of higher efficiencies, greater loading capacity and other characteristics. Nevertheless the pellicular forms will continue to have an important role since packing of columns with microparticulates in the laboratory requires experience and apparatus not commonly available; in contrast ion exchangers in pellicular form are easily dry packed. Further, microparticle packings perform optimally when the chromatographer is experienced enough to be able to fully utilise their inherent superiority. In general, then, pelliculars are used until the techniques of modern liquid chromatography are mastered. One benefit of pellicular ion exchangers is that they are relatively unaffected by sample contamination. Should the pellicular column become inoperable due to impurities in the sample, the contaminated portion of the column bed may be removed and replaced with new media.
Circle No. 41 on Reader Enquiry Coupon

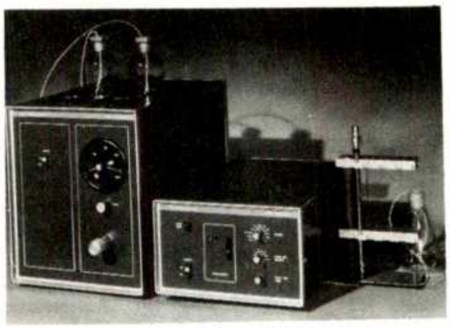
Packard introduce the 428 Series gas chromatographs which combines requirements for accuracy and reproducibility with unique analytical and instrumental flexibility. The analytical flexibility is provided through the column oven cover which contains dual injection ports, dual detectors and columns. This analytical module is a plug-in unit, thus providing the possibility to change the complete GC analysis system in minutes. Instrumental flexibility is achieved by the use of plug-in electronic modules and plug-in flow control units. Any dual detector configuration is possible, eg, dual FID, dual ECD, ECD/FID, TCD/FID etc.
Circle No. 42 on Reader Enquiry Coupon



Packard 42F Chromatograph

Pye Unicam present a wide range of general and high performance chromatography equipment. The analyst involved in repetitive routine gas chromatography analyses requires a reliable low cost, compact workhorse to achieve a steady throughput of results. The GCD chromatographs are to meet this demand.

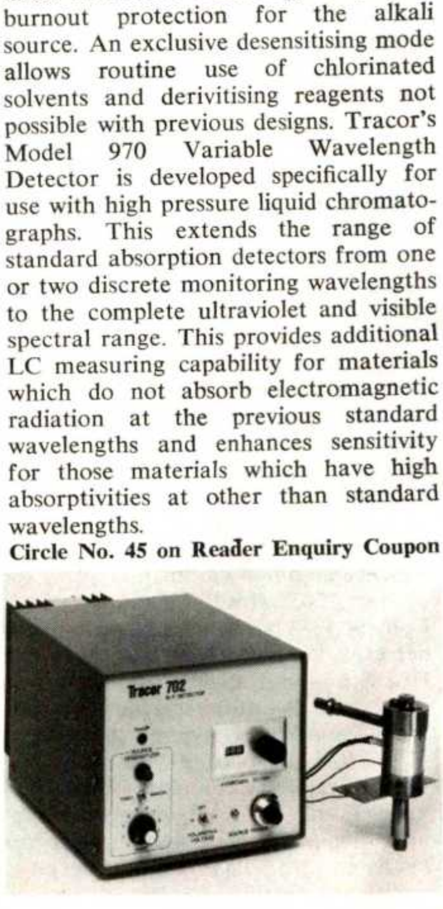
Typical applications for the GCD are to be found in laboratories engaged in multiple analyses such as quality control in the oil and gas industry, air pollution measurement and routine screening for drugs and hormones in clinical chemistry. These new flame ionisation chromatographs incorporate dual heated injection ports and dual heated flame ionisation detectors for dual column operation. Temperature control of the column oven may be chosen from isothermal or temperature programming; with conversion from isothermal to temperature programme being able to be carried out at any time. The basic chromatograph's also include a wide range ionisation amplifier, separate injector and detector oven temperature setting controls, and readout of temperature from the injectors, column oven and detector oven. The most useful accessory is the Autojector S8 which completely automates measurement of sample volume and injection of liquid samples. It is a self contained accessory which utilises punched tape to control the sequence of events that occur during the sample injection cycle. Standard tapes give facilities for six washes of the syringe before injection solvent washing of the syringe, control of integrators and identification of each analysis on the chromatogram. The sample turntable has a capacity for up to 100 samples which allows the instrument to operate unattended over long periods of time. A built-in interval timer determines the period between analyses, and may be set between the limits 0–120 min. The new Series 204 has modular construction providing a complete range of analytical gas chromatographs, starting from a simple instrument which can be subsequently extended to a fully automatic system. The basic oven assembly is suitable for single or dual column operation and includes injector, detector and column ovens all with temperature readout. A choice of five detectors is available, any two of which may be operated simultaneously. The Autojector 8 can also be integrated. The new Series 604 process chromatograph system is also geared to the less complex applications and extensions up to six streams and six components can be handled with ease and economy, due to modular extension for sample handling, analysis and programming.
Circle No. 43 on Reader Enquiry Coupon



Pye Unicam LC3

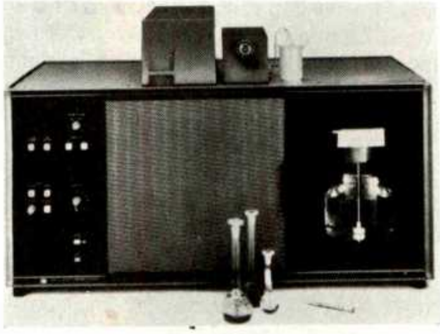
The Dickinson Instrument Company announces a Linear Gradient Maker for column chromatography and gel electrophoresis. The linear concentration gradient maker has been designed for use in column chromatography and gel electrophoresis. Mixing is accomplished by placing the gradient maker on a magnetic stirrer. Out flow is by gravity or peristaltic pump. The apparatus has a unique, leak proof valve which provides easy access, positive on-off control, with no cross hole to line up. 1,200,600 or 300 ml sizes.
Circle No. 44 on Reader Enquiry Coupon

Tracor introduces a new detector, the third generation nitrogen-phosphorous GC detector. Model 702 which combines unique mechanical design with latest electronic technology to provide burnout protection for the alkali source. An exclusive desensitising mode allows routine use of chlorinated solvents and derivitising reagents not possible with previous designs. Tracor's Model 970 Variable Wavelength Detector is developed specifically for use with high pressure liquid chromatographs. This extends the range of standard absorption detectors from one or two discrete monitoring wavelengths to the complete ultraviolet and visible spectral range. This provides additional LC measuring capability for materials which do not absorb electromagnetic radiation at the previous standard wavelengths and enhances sensitivity for those materials which have high absorptivities at other than standard wavelengths.
Circle No. 45 on Reader Enquiry Coupon



Tracor 702

Hewlett-Packard offer a new compact high performance liquid chromatograph, the HP 1081A which performs basic LC functions with an accuracy and repeatability similar to that of other members of the HP 1080 family of liquid chromatographs, but at a fraction of the price of the top-of-the-line HP liquid chromatograph. The heart of the instrument's virtually pulseless flow system is a single-head diaphragm pump whose strokes can be adjusted by a pushbutton-controlled stepping motor to set the solvent flow. The pump design assures excellent flow stability. The selected pump-stroke position and the resulting column pressure (bar) are shown on the unit's three-digit numeric display. A valve loop injector, allowing sample introduction at full column pressure without interrupting flow, comes standard with a 20-μl loop, but 10-, 50-, and 100-μl loops are also available. Other features of the flow system include a 2-l solvent reservoir with purge-gas connection and a column compartment that, although not heated, provides a protected column environment. The 1081A has a single wavelength UV detector with high signal-to-noise ratio that operates at 254 nm. The instrument, with its analog-signal output, can be linked directly to conventional integrators and strip-chart recorders. Interfacing the 1081A to a computer-based laboratory automation system is possible when an A to D converter is added. The HP 1080 Series LC family concept includes downward compatability. Analytical methods within the capability of the new 1081A can be developed on the larger, processor-based HP 1084A, the most powerful LC of the HP product line. The new methods, then, can be transferred for routine work to the smaller HP 1081A with comparable precision. The HP 5985A, with dual chemical/electron ionisation source, provides greater flexibility in measurement and data handling with such advanced features as automatic tuning, an efficient hyperbolic quadrupole mass filter, a microprocessor-controlled GC, a powerful HP 21MX E Series computer and all-digital electronics. Mass range is 10-1,000 amu and sensitiviy is to picogram levels, essential for the analysis of pesticide pollution, drug and biological samples. The instruments new inlet system is specifically designed for both packed and capillary column operation in either CI or EI mode. Sample injections may be split in a variable ratio between the GC and MS detectors to provide simultaneous quantitative GC analysis and positive identification of mass spectra in the same run. The gas chromatograph used in the system is a second-generation, microprocessor-controlled HP 5840A. This dual-column GC can be equipped with multiple detectors including dual-flame ionisation, dual-thermal-conductivity, flame-photo-metric, nitrogen-phosphorus and electron-capture detectors.


Hewlett-Packard 1081A

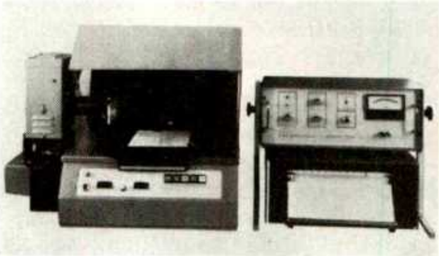
Circle No. 46 on Reader Enquiry Coupon.

Coulter Electronics Ltd introduce the Model 725 automatic injector for use with high performance liquid chromatography systems. In use it allows the automatic, unattended injection of up to 64 consecutive samples with negligible carryover between samples. The unit is microprocessor operated and it controls the number of sample injections (up to 3 per vial), injection time, rinse between sample (if desired) and automatic shut-down after completion of last sample or detection of an instrument malfunction. It uses inexpensive disposable glass vials with a unique feature. The vials caps act as a piston to discharge sample with a positive flow. Only 700 μl of sample is required and the injection valves will operate at pressures up to 6,000 p.s.i. and a 10 μl sample loop is fitted as a standard. The unit can be used with any HPLC system and it can be controlled by a computer or integrator.

Circle No. 47 on Reader Enquiry Coupon

Schoeffel introduce a new and improved, extremely-sensitive continuously variable wavelength flourescence detector for liquid chromatography. While using standard tested fluorometric technique, this new instrument offers advanced design features necessary for obtaining sensitivities required for measurements of minute fractions achieved by the superior separation capability of HPLC. Extreme care was taken to prevent fraction mixing and dead volume influence, and a unique to be efficiently collected from rather dead volume influence, and a unique 2π streadian cuvette design (5μl) resulted. This development enables light to be efficiently collected from rather weak fluorescing signals emitted from minute sample quantities (typical sample size in picogram range). The FS 970 uses a broad band deuterium (or tungsten) lamp with a high throughput monochromator to enable the user to work at the compound's absorption maximum and to assure that a wide and variable spectral range is usable. This offers the investigator greater versatility since compounds can often be excited at several wavelength. The fluorescence emission measurement system consists of a superior end-on, state of the art photomultiplier tube and a solid state photometer to offer maximum sensitivity.

Circle No. 48 on Reader Enquiry Coupon.


Schoeffel SDC300/3000

ESI Nuclear introduce a new universal radioactivity detector for gas chromatography. Light and compact, the advanced design of the new system has resulted in easy, clear controls highly suitable for the less experienced laboratory personnel. It is of great use to those involved in drug and pesticide metabolism, research or similar fields. ^{14}C labelled effluent from a gas chromatograph is passed through a furnace containing copper oxide. The ^{14}C is oxidised, passed over a drying agent, and finally counted in a gas-flow proportional counter to determine the radioactive content. Tritium compounds may also be analysed in this way by the use of an additional reduction stage containing iron filings. The new universal 505 system has a flexible heated stainless steel tube which is temperature controlled to prevent condensation of the sample in the tube connecting the gas chromatograph to the radioactivity detector.

Circle No. 49 on Reader Enquiry Coupon

Lignes Télégraphiques et Téléphoniques present microprocessor computing-integrators ICAP 10 and ICAP 5 for gas and liquid chromatography and amino-acid analysers. The two main functions are chromatogram processing and concentrations computing. They permit flexible measurement of the area of sophisticated peaks from modern chromatographs and measure, compute and visualise absolute or relative retention time. Also the micro-computing part of the systems allows the concentration calculation after introduction of response factors of each component either simple standardisation or according to an internal or external standard.

Circle No. 50 on Reader Enquiry Coupon

Laboratory Impex Ltd offers specialist equipment for the monitoring of radioisotpe tracers used in both liquid and gas chromotagraphs. The company brings together the ICN Instruments Coruflow high performance liquid scintillation detector head and its locally manufactured counting electronics and accessories to provide extremely sensitive systems for elluents containing ^{14}C, Tritium and other beta radiation emittors. The use of continuous flow monitors for radio tracers with HPLC has been made possible by improved flow cell design and detector performance and brings several additional benefits over the classical method of fraction collection and separate sample counting. The most important are that (1) sample material is almost always 100% recoverable (cf normal scintillation counting); (2) results are obtained immediately enabling experiments to proceed faster; (3) frees expensive liquid scintillation sample changers for other work.

Circle No. 51 on Reader Enquiry Coupon

Erba Science (UK) Ltd announce a series of gas chromatographs, particularly suited for capillary column applications. The current range includes: Model 2151, a well established, reliable instrument which comes complete with a FID, electrometer, oven temperature controls, carrier and auxillary gas controls, temperature programmer, Grob splitless injector and 20 m Jaeggi column Model 2351, the dual column version of Model 2151 which, in addition to the units and service outlined above, comes complete with constant flow controllers for each of the carrier gas lines, plus independent pressure regulators and pressure gauges for each of the supplies to the two detectors. Model 2900 is the newly introduced capillary dedicated gas chromatograph which may be used with split or splitless sample injectors, single or dual columns and a range of detectors. The standard specification incorporates a fast response electrometer, a large volume column oven with drop down door, digital temperature settings with readout on five points, right and left hand connections for a mass spectrometer and a heat exchanger for sub ambient operation. One of the main features of the Carlo Erba range of gas chromatographs related to capillary column work, is the lack of dead volume. This is due to an on column injection system and a unique column detector coupling which allows the column outlet to be positioned at the detector jet.

Circle No. 52 on Reader Enquiry Coupon

Techmation present, for the random access analysis of refinery gas the automatic Series-S AGC which provides multiple access analysis of the hydrocarbons and inerts of interest in various types of refinery gases. The choice of two sample valve entry points for introducing samples enables determination of components of particular or immediate interest 'out of turn' from the order dictated by a single sample sequential analysis. Four columns are used in conjunction with two multifunction gas sampling valves and a function gas sampling valves and a column switching valve. Also included for sensitive, linear measurement of hydrogen while retaining sensitivity for other components is Carle's Hydrogen Transfer System (HTS). All valve switching is, of course, automatic.

Circle No. 53 on Reader Enquiry Coupon

Erba Science IIIH

Pharmacia Fine Chemicals present a new range of high performance columns and accessories for liquid chromatography in the life sciences. All components are solvent resistant and operate at pressures up to $10\ \mathrm{kg\ cm^{-2}}$ (140 lbs per square inch). They are autoclavable and fully compatible with biological substances. The system includes colmuns SR 10/50 J and SR 10/50, columns with i.d. 10 mm and length 500 mm, with or without thermostat jacket. The columns are supplied with flow adaptors as standard. The complete range of accessories for column packing, recycling chromatography, upward flow elution and operation of columns in series consists of: column extension SRE-10, stopcock valve SRV-1; single-channel, four port valve SRV-3; two-channel, four port valve SRV-4; straight through tubing connectors SRTC-2; T-junction tubing connectors SRTC-3 and tubing end fittings for safe connection of capillary tubing.

Circle No. 54 on Reader Enquiry Coupon

Du Pont chromatographic systems have uniquely broad capabilities in areas of liquid chromatography ranging from quality control to trace analysis to gram-size preparative purifications. Instrumentation is available to operate effectively in all chromatographic modes of separation: partition, adsorption, ion exchange and steric exclusion. Modular instrument design ensures that changing analytical needs will not become obsolete in existing instrumentation. The company has incorporated the following innovations into its present range: true flow-feedback constant flow; programmable gradient elution with patented single-pump system of gradient control; simultaneous fluorescence/absorbance detection; automatic sampling; bonded packings and preparative columns for gram-size loadings. Equipment includes the 848 high performance flow controlled LC system with 833 precision controller accessory, the 838 programmable gradient electron accessory, the 830 research liquid chromatograph, and various injection systems, a variety of packings and columns; also several detectors, both fixed and multiwavelength, differential refractometers, fluorescence/absorbance detectors and the 837 spectrophotometer designed specifically for LC.

Circle No. 55 on Reader Enquiry Coupon

Varian introduces a low cost fluorescence detector for PPT trace analysis in liquid chromatography. Fluorichrom has been introduced for sensitive detection of both naturally fluorescent compounds and fluorescent derivatives. For compounds with high fluorescent quantum yields Fluorichrom can optimally measure minimum detectable concentrations (MDCs) 300 times lower than can be measured with absorption detectors. Typical Fluorichrom MDC values are: polynuclear aromatics in the 50 ppt range, amino acids, 25–75 ppt, and aflatoxins as little as 20 ppt. The photometer employs a unique dual filter system and 90° geometry to obtain low noise and high sensitivity. Two filters, rather than just one, are used at both the excitation and emission positions. The two filters reinforce one another in the cutoff regions minimising leakage and noise from unwanted light. Also, by collecting fluorescing radiation at 90° to the incident beam, noise from scattering is virtually eliminated. A broad selection of filters is available for Fluorichrom: bandpass, interference, or cutoff—for selectivity or for maximising cumulative fluorescent emission over a wide wavelength range.

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nature

25 August 1977

Believing in OSTP's merits

President Carter last month sent Congress a proposal to reorganise the constellation of offices and advisory units which together constitute his immediate White House and Executive Office staff. The proposal is the first of many promised moves to streamline and prune the federal bureaucracy, a pledge which figured prominently in Mr Carter's campaign rhetoric. For the fledgling Office of Science and Technology Policy (OSTP), the most important outcome of the proposed reorganisation is that it will be kept in business.

That fact alone signifies that OSTP has made an impression in the Carter administration during its short lifetime, for it is widely acknowledged that the people who planned the reorganisation were initially disposed to recommend that the office be disbanded. According to Dr Frank Press, Carter's science adviser and director of OSTP, the reorganisers approached the office as disbelievers, but went away as believers in OSTP's merits.

One reason for that belief is that Press has established a close working relationship with other White House units and he seems to enjoy easy access to the President, a fact which adds considerably to his standing in the White House power structure. He attends Cabinet meetings and daily meetings of Carter's senior staff, and OSTP is always represented during budget negotiations between agencies which have responsibility for R & D and the Office of Management and Budget. In addition, OSTP has been called upon to undertake a raft of studies ranging from a review of dam safety regulations to a survey of basic research policies in mission oriented agencies. Clearly, OSTP has established a solid niche.

Nevertheless, the reorganisation plan does not leave OSTP entirely unscathed. It will remove a number of functions specifically consigned to OSTP in the legislation which established the office last year, and it will result in a reduction of OSTP's staff level from the congressionally authorised 32 positions to 22. The net effect of the pruning will be to preserve and perhaps even strengthen those provisions related to the day-to-day advice to the President and other White House units, while stripping away some responsibilities for analysing longer term issues.

Although those losses may seem like a small price to pay for retaining OSTP in the White House, they are cause for concern. In fact, there has recently been some grumbling from OSTP's chief sponsors on Capitol Hill, notably Olin Teague, chairman of the House Committee on Science and Technology, and Senator Adlai Stevenson, chairman of the Senate Commerce Subcommittee on Science, Technology and Space. Both have sent letters to the chairmen of the House and Senate Government Operations Committees, which will be reviewing the reorganisation plan, expressing concern at the potential loss of some of OSTP's functions.

Specifically, the reorganisation plan would relieve OSTP of the responsibility for preparing two reports, a five-year outlook identifying problems and issues likely to require attention in the years ahead, and a series of annual reports reviewing developments in science and technology and assessing selected government programmes. Both those tasks will now be undertaken by the National Science Foundation, which is probably as well equipped as OSTP to write such reports, but studies carrying a White House imprimatur would carry much more weight.

The reorganisation plan would also do away with the President's Committee on Science and Technology (PCST), a top-level advisory committee similar to the former powerful President's Science Advisory Committee. PCST was established by Congress primarily to conduct a two-year review of federal science policies and to recommend organisational changes in the federal bureaucracy dealing with science and technology. At the end of PCST's review, Carter would have had the option of keeping the committee as a permanent advisory body.

The committee will be scrapped chiefly because a White House unit is already looking at government reorganisation—having a separate committee looking into the organisation of science agencies was deemed superfluous. Nevertheless, the committee's demise will remove a potentially important source of outside opinion particularly on longer term issues which lie beyond the day-to-day matters facing the White House.

When Congress created OSTP last year, it gave it the dual role of advising the President and other White House units on issues involving science and technology, and of providing long range analysis of broad questions concerned with science and social policy. The reorganisation seems to have strengthened the first role by ensuring that the Science Adviser will have a secure place in the upper echelons of the White House. But the second function has been downgraded. □

Science in the Himalayas

Peter D. Moore, recently in India, finds some scientific surprises in the mountains

ANYONE who travels in India soon grows accustomed to the unexpected. Even so, the little town of Dehra Dun, nestling in the foothills of the Himalayas ten hours' train journey north of Delhi, will surprise the most hardened traveller, for it occupies an influential position in Indian science. It serves as a centre for research, training and exploration in such industries as oil and natural gas and in forestry, and it is also the base for the Northern Region of the Botanical Survey of India.

The most unexpected of these is perhaps the oil industry, especially when one considers the distribution of oil and natural gas exploitation in India. At present there are two major sedimentary basins yielding oil (see accompanying map), located at the eastern and western extremities of the subcontinent. In the east is the Assam-Arakan basin, which is being exploited by both the Indian Oil and Natural Gas Commission (ONGC) and by Oil India Ltd, in which the Indian Government and the British Burma Oil Company hold equal shares. At present Oil India is producing 3.08×10^6 tons annually from Assam, representing more than a third of India's total oil production. Further development of this field could double its output. In the west of India, in Gurarat, is the Cambay Basin, which extends from the coast to the gas-bearing strata of Rajasthan and south into the Gulf of Cambay. This field was discovered in the late 1950s and since then the offshore fields of the Bombay High have also been brought into production.

Crude oil production has risen from 259,000 tons a year in 1951 to a present output of about 8×10^6 tons. Greatest expansion was achieved between 1963 and 1969. At the same time annual consumption of petroleum products has risen from 3.9×10^6 tons in 1951 to a present level of about 23×10^6 tons. India is thus producing only one third of her oil needs.

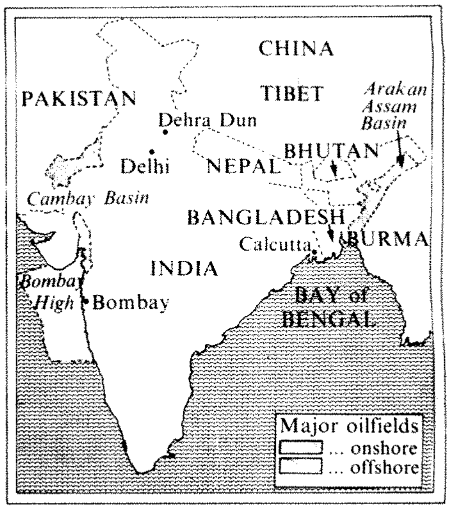
Oil consumption in India will undoubtedly continue to grow. Apart from her fuel requirements, the growth of the petrochemical and fertiliser industries will present an increasing demand. There is already talk of lining canals with polythene to prevent water losses, and even the possibility of covering whole fields. In fishing, clothing and domestic construction, particularly in the improvement of water supplies and sewerage, plastic products will contribute to efficiency and to health. Historically, many apparently profound influences have been passively absorbed by India and have been swallowed by the inertia of her ageless culture, leaving her unchanged. Will plastics really alter the face of India? One wonders whether the Indian's concern for the welfare of bovines will actually result in a plastic bullock cart fitted with pneumatic tyres—a suggestion recently made by Dr S. Varadarajan, Chairman of Indian Petrochemicals Ltd.

Growing demand

On the fertiliser side, the current production of about 1.5×10^6 tons of nitrogen has placed a growing demand upon crude oil. Many plants consume sulphur-rich fuel oil, which can be imported relatively cheaply because of the air pollution resulting from SO_2 production on combustion and because of the strength of the ecological lobby in the industrialised nations. India, like so many developing nations, does not yet feel in a position to afford the clean air which the developed nations now vainly seek.

What India also cannot afford is that its oil import bill should continue to rise. Great emphasis is now placed, therefore, upon the closure of the gap between home production and consumption of petroleum products. Optimistic predictions abound in India at present. Prospects for the development of existing fields, particularly the offshore drilling in the Bombay High, are good. Proven reserves on land in India are estimated at 700×10^6 tons, of which 200×10^6 tons are considered recoverable, while the Bombay High is thought to contain at least another 800×10^6 tons. N. B. Prasad, chairman of ONGC, considers that production from these major fields can rise to about 20×10^6 tons a year within the next four years, thus narrowing very considerably the gap between production and demand in the country.

The political dream of an India self-supporting in her oil needs, however, still demands further exploration and development. This is where the Himalayan town of Dehra Dun comes into the picture. In 1963 an Institute of Petroleum Exploration was set up with the aid of money from the United Nations Development Programme. The apparently strange choice of site (almost equally distant from both of the major oil producing areas) can be explained by the contemporary search for oil in the Himalaya foothills. In the fourteen years since the inception of this institute no reserves of a commercially viable nature have yet been discovered, thus leaving it stranded in a remote but scenic location which was once the summer playground of the British Raj.



Exploration and analytical research remain the prime functions of the Dehra Dun Institute. A comprehensive evaluation of the sedimentary basins of India is under way, no mean task given that it covers basin areas of 10^6 km² onshore and 320,000 km² offshore. The volume of chemical, geological and palynological data generated in the laboratories at Dehra Dun has necessitated the installation of an IBM 370/145 computer in the institute for rapid assessment of oil-bearing potential. Russian influence at the Institute has been and remains strong. The original UN representative at the establishment of the institute was a Russian and a team of scientists from the Soviet Union assists in research and analysis.

Forestry too

The Institute of Petroleum Exploration is not the only surprise awaiting the visitor to Dehra Dun. Just 6 km west of the town stands a most extraordinary and impressive building set in 445 ha of lawns and gardens. It houses the Indian Forest Research Institute. Built in 1929 and inaugurated by the then Viceroy of India, Lord Irwin, this institute is a monument to the economic importance of forestry in India. It began as the Imperial Forest School in 1884 and then expanded to encompass forest research. It now serves both a research and teaching function and houses a staff of 1,900.

As in many other countries, India has suffered badly from deforestation and the resultant soil erosion. This has reputedly come about particularly since Moghul times in north India. It is said (usually by Hindus) that the Mohammedans did not possess the same respect for vegetation as their Hindu predecessors. It is certainly true that vast areas of the Himalayan foothills have been stripped of forest and now only fragments remain. This has come about both as a result of intensive grazing and also the use of wood for fuel.

The varied topography in the region of Dehra Dun provides an ideal setting for a Forestry Institute. The steep slopes of the Lesser Himalayas take one up out of the sub-tropical climate of the plains and the Siwalik Hills into the cool temperate climate of the foothill ridges. Broad features of forest zonation accompany these altitude changes. It is thus possible for students at the Forest Research Institute to gain experience in a variety of forest conditions.

Besides the practical aspects of silviculture, the institute offers training and research facilities in such fields as entomology, paper technology, wood physics, timber mechanics and genetics. The main buildings house a vast museum and herbarium. The institute has served an important function in the development of techniques suitable for the conditions found in India. For example, the use of bamboo as a raw material in the manufacture of paper was pioneered here, as was the use of sugar cane waste. Bamboo, the poor man's timber and the standard material for scaffolding in India, represents a jungle to the taxonomist. There are about a hundred species and they flower only once, immediately prior to death, so that by the time you can identify them it is too late. Techniques for identification on the basis of culm sheaths and young bud structure are currently being worked upon, since identification is essential for the further development of bamboo management methods.

Besides the obvious products of forestry, timber and pulp, the Indian forests are rich in species which are economically important as a source of minor products. For example, the oil from sandalwood is an important earner of foreign exchange for India. The production of santonin from certain *Artemisia* species is an area which has recently received attention. The survey of Indian plants for potentially useful species is thus of considerable importance. The Forest Research Institute deals with woody plants, but what of the others?

Botanical survey

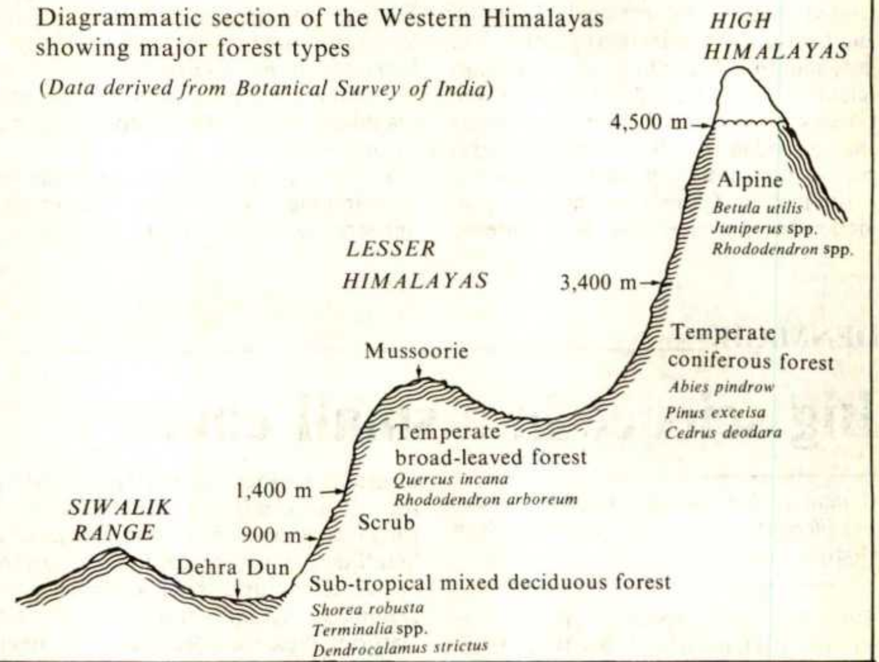
It comes as no surprise to find yet another institute tucked away in the suburbs of Dehra Dun, this time the headquarters of the Northern Circle of the Botanical Survey of India. This is a government organisation which is centred on Calcutta and which functions through four Regional Circles. The emphasis is upon plants as a national resource (in an economic rather than an aesthetic sense), but the bulk of the work is purely taxonomic. At present the flora of India has still not been fully described and the high Himalayas represent a particularly underworked area.

Because a major aim of the survey is the discovery of plants with a commercial potential, especially those with medicinal properties, the development of a herbarium of dried specimens is not enough; plants must be kept alive. The establishment of a botanical garden is therefore a priority and, as many of the more interesting plants derive from high altitude, the garden must also be sited in the hills. The British developed an upland botanic garden at Mussoorie (about 2,200 m) but this has now been abandoned as a scientific venture. A new garden has been established at Pauri (1,500 m) which will be used to maintain populations of high altitude plants and for experimentation. Such a venture may also prove to be valuable for the conservation of some rarer species, such as some of the orchids, and may also provide material for horticultural developments.

Perhaps the plant resources of the Himalayas will soon provide the basis for further commercial exploitation in the form of tourism. It still takes a considerable muscular effort to reach the Bhyundhar Valley (the 'Valley of Flowers') at 3,600 m but one can envisage changes coming about as India seeks more opportunities to earn foreign exchange. Although the Hindu pilgrims are prepared to trek on foot to their mountain shrines, the Western tourist will undoubtedly demand the development and despoilation of these remote areas. Conservatism, a right sense of priorities, or even sheer inertia may make India unwilling to pay the price. ☐

Diagrammatic section of the Western Himalayas showing major forest types

(Data derived from Botanical Survey of India)



Cultivated terraces near Mussoorie in the Himalayas

EARTHQUAKES

Warning in time

Accounts of recent successful earthquake predictions in China have reached Western scientists. Colin Norman reports

THREE successful earthquake predictions were made in China last year, and warnings were issued in time to evacuate people from hazardous buildings in the threatened area. But there were also some failures. A number of false alarms were issued, and Chinese scientists failed to predict the devastating earthquake which levelled the city of T'angshan in July last year.

Those recent successes and failures were discussed for the first time by Chinese scientists who attended an international meeting on earthquake prediction sponsored by UNESCO (the United Nations Educational, Scientific and Cultural Organization) in Paris late last month. According to American scientists who attended the meeting, the Chinese were frank in their discussions and provided much previously unreported information on their methods.

Interest in China's earthquake prediction programme has been intense ever since it became known in the West that Chinese scientists had accurately predicted a major earthquake which struck the city of Haicheng in February 1975. That prediction led to evacution of the city and it is credited with saving many lives.

The latest successful predictions, according to Robert L. Wesson, a seismologist from the US Geological Survey who attended the UNESCO meeting, involved three earthquakes in 1976 of about magnitude 7 on the Richter Scale. They occurred in Yunnan Province on 29 May, in Szechuan Province on 16 August, and in the Szechuan–Yunnan border region on 7 November.

Wesson reported that in each case, Chinese scientists made long-term predictions that an earthquake would occur. Based on a variety of geophysical and magnetic anomalies, the long-term predictions were used as a basis for more intense monitoring in the threatened area, and for disaster planning. They were, however, not made public.

Public warnings that an earthquake was imminent were issued only on the appearance of foreshocks—minor tremors which sometimes precede major quakes. The public warnings, according to Wesson, were accompanied by evacuation of people from potentially hazardous buildings. The appearance of foreshocks in the Haicheng area was also used as the trigger for issuing an earthquake warning there in 1975.

Major earthquakes frequently strike abruptly, however, with no foreshocks, and thus imminent hazard warnings may not be issued in time. That seems to have been the case with the T'angshan earthquake. Wesson said that the Chinese had made a medium-term prediction of a quake in the area which had underestimated the magnitude of the event and no imminent warning was issued. In the past, Chinese officials have been reluctant to discuss the T'angshan earthquake, which killed more than 600,000 people.

Wesson also said that the Chinese scientists at the UNESCO meeting admitted that several false alarms have been issued, although their success rate was not revealed. According to Dr Jack Savage, a geophysicist who has visited China, Chinese scientists have acknowledged issuing false alarms before, but they argue that they would prefer to issue warnings whenever necessary rather than risk the consequences of an unpredicted major quake. □

DENMARK

Big science in a small country

A new nuclear physics project is under consideration in Denmark. Sven Godtfredsen reports

THE fate of experimental nuclear physics at Denmark's Niels Bohr Institute is in the process of being decided. An accelerator project now under consideration by the Ministry of Education marks an attempt by the institute to proceed on its own following the failure of an attempt at Scandinavian collaboration. But the enormous costs mean that the matter is likely to become a central issue in Danish science policy.

The project, known as the Danish heavy ion project, was submitted to the Ministry of Education, by the Danish Council for Scientific Policy and Planning. It involves building a 20MV or 25MV tandem accelerator. The total cost of the project will include the cost of building the accelerator, estimated at DKr115 million or DKr86 million depending on the choice of terminal voltage, and the DKr3 million increase in grants to the institute for operating the accelerator (£1 = DKr10.4). Financially speaking, the project is breathtaking compared to expenditure on other natural science projects in Denmark. Grants from the Danish Natural Science Research Council (DNSRC) whose function is to finance and to some extent lead Danish research in the natural sciences, amounts to only DKr25 million a year.

The DNSRC gave its view on the project last May in a report called *The Future of Nuclear Physics in Denmark*. Apart from examining the scientific content of the heavy ion project, the report reviews it in relation to broader issues—specifically, Danish participation in CERN, the attempts to establish collaboration in nuclear physics between the four Scandinavian countries (the NORDAC project), the possibility of collaboration between the institute and the heavy ion laboratory in Darmstadt, and of course, the particular financial aspects of nuclear physics in Denmark. But the question really concerning the DNSRC is how long a small country like Denmark can run its own programme in such an expensive branch of natural science.

This question is not new. It was asked in 1970 when the Niels Bohr Institute needed to renew the 9 MV tandem accelerator, and inspired nuclear physicists from the four Scandinavian countries to discuss the possibility of building a major heavy ion laboratory in the North. The idea was elaborated into the NORDAC project, which the DNSRC strongly recommended and which received financial assurances from the Danish government. Unfortunately the other Scandinavian countries were unable to back the project. Since then the institute has used the heavy ion tandem accelerator in Darmstadt for most of its nuclear physics experiments. But scientists at the institute feel that this approach weakens the contact between experimental and theoretical nuclear physics, traditionally the institute's strong feature.

There is little reason to believe that the NORDAC project is any more likely to happen now, so the Niels Bohr

Institute has decided to try to proceed on its own. Once again it has obtained full support from the DNSRC which argues that the proposed accelerator must be a national as well as an international asset. Current research at the institute needs an accelerator which provides a beam of very high energy heavy ions with good ionoptic properties and of very precise energy which can be varied rapidly. At present these requirements are only fulfilled by heavy ion tandem accelerators three of which are currently under construction: at Oak Ridge, Tennessee, in the USA (25 MV), at Daresbury in Britain (20 MV, which can be upgraded to 30 MV) and at the Atomic Energy Research Institute in Japan (20 MV). The DNSRC believes that the kind of nuclear physics research which can be undertaken with these accelerators is so fruitful that more accelerators than these three instruments will be needed in the near future.

Both the DNSRC and the Danish Council for Scientific Policy and Planning stress that the accelerator is required to continue the close interplay between theoretical and experimental work and "to sustain the status of the Niels Bohr Institute as a centre of excellence". Without the 25 MV or the 20 MV tandem they believe the institute will stagnate; with it they believe the institute will use its capabilities to provide results "with consequences for the world picture presented by modern physics". □

COMECON

- The thirty-second Session of the Comecon Permanent Commission for the Peaceful Uses of Atomic Energy which took place recently in Budapest discussed the long-term draft plan for fuel, energy and raw materials, cooperation in the use of radiation in industry, agriculture and environmental protection, the improvement of anti-radiation precautions and protection, and the disposal of radioactive waste. Also considered was the significance of cooperation in nuclear power for the overall coordinated plan for integrating the member countries' economies. To celebrate the thirtieth anniversary of Comecon, the Commission will publish, in 1979, a book entitled *The Peaceful Atom in the Countries of Socialism*.

Long-term energy and raw materials needs was also one of the themes of the recent meeting in Havana of the Permanent Commission on Power. In addition to discussing the long-term development of the unified power grids of the continental members of Comecon, special attention was paid to the particular needs of Cuba. A broad programme of new investments includes the construction of the first nuclear power station and the installation of transmission lines of higher tension than the present 220V. Cuba is already involved in a number of Comecon bilateral agreements for cooperation in nuclear and conventional energy with the USSR, East Germany, Hungary and Poland. According to J. L. Beltrami, the Cuban Minister of the Electrical Industry, it hopes to conclude similar agreemnts with the other member countries in the near future, and to establish a programme of multilateral cooperation.

- A new Comecon international organisation, Agroinform, has been established to coordinate scientific and technical information on agriculture and forestry. It is part of a general policy of integrated information systems for the Comecon bloc, based on the International Scientific and Technical Information Centre of Moscow, to coordinate the activities of member countries in collecting, selecting and processing information. So far Agroinform has dealt mainly with details of organisation and administration; this autumn, however, its International Council will meet in Prague to discuss the system's technical details. For the present exchange of agricultural information remains largely a matter of bilateral negotiations, such as the recent agreement between the Polish Academy of Sciences and the Czechoslovak Federal Ministry of Agriculture and Food covering research into the genetic and environmental factors affecting the quality of meat.

- During the past few years, Czechoslovak industry has made massive capital investments in plant and machinery purchased from abroad. By the end of 1976, some 15,000 million Czechoslovak crowns had been spent on equipment from 'capitalist' countries, a sum which theoretically is to be recouped by ever-increasing exports. But unfortunately, some 58,000 million crowns worth of the imported equipment is still standing idle, due to delay in the construction of factories to house it. At one chemical plant, Chemiceluloza, 200 million crowns worth of imported equipment is reportedly waiting at the construction site and cannot be installed until late in 1978.

- A second Polish Antarctic research station will come into operation during the coming southern summer. Named the 'Antoni Boleslaw Dobrowolski' station, it was handed over to Poland by the Soviet Union in 1959 but has not been used until now because of a lack of funds. Situated at the Bunger Oasis, 300 km from the coast and 4,000 km from the existing Polish 'Henryk Arctowski' station on King George Island, it will conduct mainly geophysical and meteorological research sponsored by the Geophysics Institute of the Polish Academy of Sciences.

The opening of Dobrowolski station is part of a general increase of Polish activity in the Polar regions. After an intensive programme of geophysical, meteorological and oceanographic research at the Arctowski station last summer, a team of 19 scientists manned it for the present winter. Meteorological data from the station forms an important link in the weather-forecasting network based on the Soviet Molodezhnaya station.

- A detailed programme has been worked out for joint Comecon research into the use of solar energy. The programme is largely based on Soviet experience in this field; at present, about 100 small solar power stations are operating in the Soviet Union, and are expected to operate without interruption for more than 10 years. According to the Soviet newspaper *Trud*, these stations have one great disadvantage—the high cost of the electricity produced.

Vera Rich

IN BRIEF

JET suffering

The European Community's Joint European Torus (JET) fusion project, now costed at almost £100 million at January 1977 prices, is "overdue" and "suffering from serious lack of decision on vital matters", according to Bas Pease, the director of the Culham Laboratory near Oxford, where the JET design team is based. Writing in the laboratory's 1976 annual report, Dr Pease says that prospects of future European international cooperation depend on confidence that technically sound projects like JET can be carried out. The report also says that uncertainties over the site have made it difficult to plan certain work on JET.

Marine responsibilities

Those who believe that very few subjects are the specific responsibility of a single government department in Britain are vindicated by the contents of a publication prepared by the UK Government's Interdepartmental Committee on Marine Safety. Entitled *Marine Activities. Guide to the responsibilities of government departments and agencies,* the booklet shows, that marine pollution involving oil discharges, is the responsibility of the Department of the Environment when the discharges are from land or offshore installations within the 3-mile limit, but fall under the Department of Energy outside that limit. Occupational health and safety are the responsibility of the Health and Safety Commission. The booklet is published by the Department of Trade

IAEA's report

The growing uncertainty over the future of nuclear power in some industrialised countries is reflected in the International Atomic Energy Agency's annual report for 1976. It was partly responsible, the report claims, for orders for new nuclear plant falling from 53,000 MW in 1974 to 11,000 MW in 1976. The IAEA's total estimated regular budget for 1978 is $48.8 million (£1=$1 74). Expansion is planned for some areas of its work.

Frugal farming?

KENNETH MELLANBY

WITH the possibility of a world shortage of energy within the next twenty five years, many people are concerned lest this should jeopardise our food supplies. They are told that modern farming is 'energy intensive', producing fewer calories to eat than are expended in their production—in cultivations, manuring and harvesting—notwithstanding the stored solar energy obtained by photosynthesis.

It is sometimes therefore suggested, particularly by some who call themselves 'ecologists', that we should return to more primitive farming systems, or even to the practices of the pre-agricultural hunter and gatherer. The bushmen of the Kalahari desert in South Africa are often cited as examples of global frugality, living with the minimum disruption of the natural ecosystem, and taking out much more energy than they put into it. It is generally forgotten that this type of life is only possible with small and sparse populations; it is thought that the whole of Britain could only feed some five thousand people before the introduction of arable farming. The whole world might support about a million.

Without going so far back, it is perhaps worth looking at some of the farming methods used fifty or a hundred years ago. Thus if oil is getting scarce, it may be logical to use more horses. An analysis of the effect of such a change gives the surprising result that there would be an increase, not a decrease, in energy consumption.

In Britain today all the tractors, combine harvesters and other farm machinery use about 1% of our total oil consumption (under one million tons coal equivalent), even allowing for the fuel consumed in the factories making the machines. In 1939 we had about a million working horses on our farms, but we would need at least double that number to harvest the vastly greater crops grown today. These horses would need to be fed every day of the year, not (like a tractor) only when they were working. It used to be said that a horse needed three acres of land for its support, but much of this was probably rough grazing. Nevertheless they would need some grass, and possibly as much as two tons of oats, or its equivalent in other grain, per horse per year. This would mean that more than two million acres of the best farm land would be needed to support the horses, and so would be lost for the production of human food.

The energy equation is equally disastrous. The calorific value of the horses' food would be at least four times that of the fuel powering the modern tractors, which do the work so much more quickly, and so enable the farmers to take advantage of Britain's changeable weather. In a bad year it is likely that, working with horses, much of the harvest would never be gathered in, and the land would seldom be cultivated in time for the next sowing.

Then if we went back to horse power, the human workforce would need to be doubled or trebled. Some people would welcome this, believing that it might decrease unemployment and reinvigorate village life. But are we prepared to pay all these farm workers the sort of wages they would expect from industry, when there would be a fall, rather than an increase, in food production? And where would they live? We have virtually abolished the 'tied cottage' reserved for farm workers; the houses are occupied by commuters, the retired and weekend visitors. On a purely arable farm the employees can travel to work, but where animals, including horses, are kept, the staff must live close at hand.

The main reason modern farming appears to use so much energy is not its machinery, but the inefficient food conversion of its intensively kept livestock, which waste over 90% of the food they eat. Our farm animals consume about forty times the energy used by our tractors. Horses, like other animals, are poor converters of energy. The surprising thing is that our highly mechanised farming techniques in Britain use only some 3% of our total energy budget. If yields are to be maintained, a return to old techniques and to the use of horses would greatly increase the input of energy down on the farm.

news and views

The return of whole-mantle convection

from Peter J. Smith

'THOUGH it has been clear for ten years that the motion of plates must involve flow in the mantle below, nothing is yet known with certainty about the form this motion takes.' This recent remark from McKenzie (*Geophys. J.*, **48**, 211; 1977) is indisputably true but conceals some interesting swings of opinion. When mantle convection was first proposed by Holmes (*Trans. Geol. Soc. Glasgow*, **18**, 599; 1931) and others it was clearly envisaged as a process taking place throughout the whole of the region between the crust and core. By the 1960s, however, when the hypotheses of continental drift and seafloor spreading were being combined and extended into the theory of plate tectonics, the idea of whole-mantle motion had already fallen into considerable, though not complete, disfavour. Some Earth scientists had produced what they felt were valid reasons for supposing the lower mantle to be immobile, and others had greatly increased our knowledge of the thin mobile layer immediately below the lithosphere. It was perhaps inevitable, therefore, that the asthenosphere should come to be regarded by many workers as the key to the lithospheric movements above.

But a year or so ago the idea of convection throughout the mantle (or, more correctly, throughout that part of the mantle below the lithosphere) began to regain favour; and this trend continues. Gough (*Earth planet. Sci. Lett.*, **34**, 360; 1977), for example, has come down in favour of whole-mantle convection on the basis of a study of the geoid. When departures of the geoid from the flattened Earth spheroid are plotted on a sphere, a single zero-elevation contour is found to divide the surface into two nearly equal zones with positive and negative elevations respectively. Moreover, these two zones are interleaved rather like the strips which cover a tennis ball. The areas covered by the zone of positive elevation include the western Pacific, most of Australia, the Arctic, the North Atlantic–European region, western South America, the South Atlantic and the South Indian Ocean. The areas covered by the zone of negative elevation, on the other hand, include much of Asia and the Indian Ocean, the Antarctic, North America, the Brazilian shield, the eastern Pacific and the western Atlantic.

What does this antisymmetric pattern mean? To Gough it suggests mantle-wide convection, the scale of the two zones being too great to be attributable to the effects of near-surface structure. As Gough envisages it, upward currents in the mantle would exist beneath all areas of negative elevation (low geoid), there would be downward currents beneath areas of positive elevation, and horizontal flow would occur across the zero-elevation contour (one way near the surface and the other way at the base of the mantle). In other words, by this interpretation the geoid represents 'whole-mantle convection in a single three-dimensional cell.'

If this is right, it then raises the question of the extent to which mantle-wide convection influences the motion of the lithosphere. Gough sees some 'encouraging relationships' between his model and actual plate behaviour; for example, the model predicts rising currents beneath the East Pacific rise and downward flow beneath the western Pacific subduction zones. On the whole, however, he is inclined not to expect close correlations between global convection and plate motion, arguing that lower mantle behaviour will be partially decoupled from the lithosphere by the intervention of the asthenosphere. In other words, the existence of mantle-wide convection would not preclude shorter wavelength convection in the asthenosphere with perhaps greater influence on the overlying plates.

But this rather defensive posture is not shared by Davies (*Geophys. J.*, **49**, 459; 1977) who claims it to be 'unlikely that the lower mantle is *not* involved in motions related to plate tectonics.' Davies, too, draws attention to the possibility of small-scale flow in the upper mantle but he clearly regards such motions as perturbations on large-scale mantle-wide convection, thus imputing to them relatively less importance than does Gough. It would be misleading to make too much of this difference in emphasis, for both authors share the conviction that the limitation of convection to the upper mantle is unlikely. Davies is able to be somewhat more positive, however, because his conclusion is based on the study of whole-mantle convection models which, although simplified, can account qualitatively for the large horizontal scale of plates, the imperfect correlation between plate speed and the amount of attached descending slab, the motion of plates with no attached slab, and the opening of the Atlantic in particular.

What Davies's study of simple layered-fluid models shows is that thermal convection would be confined to the upper mantle only if the lower mantle were at least 10,000 times more viscous than the upper mantle. And even if the mantle were to play only a passive part in plate dynamics, the flow induced by the moving plates would fail to penetrate the lower mantle only if the viscosity of the lower mantle were at least 1,000 times greater than that of the upper mantle. Moreover, a shallow low-viscosity layer would have to be about 10,000 times less viscous than the mantle beneath for flow to be confined to it. In other words, it would appear that mantle-wide flow, whether induced thermally or by moving plates above, can only be prevented by extreme viscosity contrasts in the mantle.

Traditionally, of course, such contrasts have been widely accepted, or, as Davies puts it, 'speculations on and

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models of mantle flow associated with plate tectonics have been dominated by the concepts of an elastic, high-viscosity lithosphere, a relatively low-viscosity asthenosphere, and a high-viscosity mesosphere.' Indeed, as far as the high-viscosity lithosphere and lower-viscosity asthenosphere are concerned, the tradition persists; the existence of these features is not seriously in doubt. What is at issue, however, is the viscosity of the lower mantle and its contrasts with, on the one hand, the viscosity of the asthenosphere and, on the other, the viscosity of the rest of the upper mantle below the asthenosphere.

To cut a long story short, several reasons have been adduced in the past for supposing the viscosity of the lower mantle to be many (for example, five or more) orders of magnitude greater than that of the asthenosphere, a view which appeared to have majority support until quite recently. But as Cathles's extensive studies have shown (*The Viscosity of the Earth's Mantle*, Princeton University Press, 1975), there are apparently equally good reasons to suppose that the contrast is much less. Cathles's own version is that the mantle has a fairly uniform viscosity of about 10^{22} poise throughout, apart from a thin channel (the asthenosphere) in the uppermost mantle which has a viscosity of about 0.4×10^{21} poise. It is difficult to say whether or not this view of mantle viscosity has become the new consensus; what can be said without fear of dispute is that the low-contrast mantle is now more popular than it was a few years ago and that it is now more fashionable to debunk arguments in favour of the high-viscosity lower mantle.

This clearly strengthens the case for mantle-wide convection, especially in the light of Davies's results. But what of the other traditional arguments against whole-mantle convection? In a very useful review section Davies looks at all the main ones and concludes in the light of recent information that none is of compelling validity. It has been suggested many times in the past, for example, that there may be chemical composition gradients in the mantle involving changes in mean atomic weight—obviously a situation which would be difficult to reconcile with the mixing brought about by whole-mantle convection. But as Mao (*J. geophys. Res.* **79**, 5447; 1974) and others have shown, the particular observations which led to the invocation of atomic weight changes can be explained at least as reasonably in other ways. Obviously this does not rule out the existence of chemical non-uniformity but it weakens the ability of such non-uniformity to stand as an argument against mantle-wide convection.

The evidence for the presence of phase transitions in the mantle, on the other hand, is much stronger, which raises the questions of whether phase boundaries can persist in the face of convective mixing and of whether the persistence of such boundaries may prevent convective flow across them. The available information here is conflicting, for much depends on the precise conditions in the mantle. Davies concludes, however, that the case for saying that phase transitions will prevent whole-mantle convection is suspect at best, although such transitions could well modify the pattern of convective flow. Likewise, the case for excluding the lower mantle from involvement in plate dynamics on the grounds, among others, that earthquakes do not occur below about 700 km is also dubious, for there are ways of explaining this lack of seismicity without assuming that descending lithosphere fails to reach beyond this depth.

In summary, then, Davies has presented what is probably the most convincing case yet in favour of convection throughout the mantle below the lithosphere, or at least the most convincing case for not rejecting whole-mantle convection quite so easily as it has often been rejected in the recent past. Nor is he alone. The support from Gough has already been mentioned, but there is more. Jones (*J. geophys. Res.*, **82**, 1703; 1977), for example, has suggested that long-term variations in the frequency of reversal of the geomagnetic field may be the result of fluctuating temperatures at the core-mantle boundary caused by convection in the lower mantle. And perhaps more importantly, Dziewonski *et al.* (*J. geophys. Res.*, in the press) are about to show that the large-scale seismic velocity anomalies detected in the mantle, including the lower mantle, by Niazi (*Bull. Seismol. Soc. Am.*, **63**, 2035; 1974) and others correlate with long-wavelength components of the gravity field—an observation which O'Connell (*Tectonophysics*, in the press) will relate to mantle motions. It would seem that the few people (for example, Runcorn) who have maintained their belief in mantle-wide convection for many years, if not decades, are to be vindicated, or at least are to see their views become fashionable again. ☐

Bed bugs and hepatitis

from Arie J. Zuckerman

ALTHOUGH much has been written during the past few years on all aspects of human viral hepatitis, and hepatitis B in particular, it seems that this infection still has some epidemiological surprises to spring. The latest is a report by Willis *et al.* (*Lancet* **ii**, 217; 1977) that the bed bug could be a vector of hepatitis B.

With some 120–176 million carriers worldwide, the importance of hepatitis B cannot be overemphasised. In regions such as northern Europe, North America and Australia, where infection is relatively uncommon (a carrier rate of ~ 1 in 100), transmission is chiefly through inoculation of blood and blood products, although hepatitis B can also be transmitted sexually and in other body fluids such as saliva, menstrual discharge, breast milk and semen. In areas where infection is relatively common such as parts of tropical Africa, South East Asia and the Far East (with a carrier rate of 10–20% of the population), transmission by direct inoculation of blood or blood products is not enough to account for the higher rate of infection. The modes of transmission of hepatitis B in the tropics are similar to those in other parts of the world, but additional factors may be of importance including traditional tattooing and scarification, ritual circumcision with unsterile instruments and repeated biting by blood sucking arthropod vectors, and in particular mosquitoes (or flying-pins; *Nature* **239**, 72; 1972) and bed bugs.

Review of the available evidence on the role of biting insects in the spread of hepatitis B shows that the results of preliminary investigations are conflicting. Hepatitis B surface antigen, which is one marker of the virus, has been detected in several species of mosquito, which have been trapped in the wild or fed on infected blood artificially, but no convincing evidence has been obtained either of replication of the virus or of its persistence after digestion of the blood meal. The very real possibility of mechanical transmission by mosquitoes nevertheless exists, for example as a result of interrupted and consecutive feedings particularly in areas with a high prevalence of hepatitis B (*Tech. Rep. Ser. Wld Hlth Org.*,

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The bed bug, *Cimex lectularius* which lives in cracks and crevices in the house, especially in beds.

Photo: C. James Webb, London School of Hygiene and Tropical Medicine.

No. 602; 1977). Bed bugs live more intimately with man than do mosquitoes, take blood meals and could transfer blood and hepatitis B virus from one occupant of a bed to another. Brotman and her colleagues (*Lancet* **i,** 1305; 1973) collected bed bugs from dwellings occupied by prostitutes in the Ivory Coast and detected hepatitis B surface antigen in 1 (5.6%) of 18 pools of engorged bed bugs of the species *Cimex hemipterus.* In a laboratory study, Newkirk *et al.* (*Gastroenterology,* **69,** 982; 1975) artificially fed *Cimex lectularius* bed bugs on blood from a patient with acute hepatitis B. Hepatitis B surface antigen remained detectable in the bed bugs for over 4 weeks, and juvenile bed bugs contained antigen after moulting, at a time when the bug usually starts to search for a host and will refeed. In the course of another investigation, carried out in Senegal by Wills *et al.* (*op. cit.*) bed bugs were collected on four separate occasions from bedding in the huts of villagers. All the bed bugs were identified as *Cimex hemipterus,* the predominant species in West Africa and other tropical areas. Hepatitis B surface antigen was detected in unengorged nymph and adult bed bugs in each of the first three collections. Three out of 28 specimens were antigen-positive in the first collection and 3 of 17 specimens were positive in the second collection. In the third collection, 6 out of 9 bed bugs were positive when the bed occupant was known to be hepatitis B surface antigen-positive. The antigen was also detected in 3 out of 89 bed bugs of the fourth collection kept alive without a blood meal for 30 days. Of particular interest was the finding of the *e* antigen, which is a marker of infectivity of hepatitis B virus (see *News and Views* **263,** 374; 1976), in 1 engorged and 1 unengorged bed bug. These newest reports are the highest field infection rates with markers of hepatitis B virus reported so far in any insect species. Clearly bed bugs feeding on the occupants of the same bed could amplify the risk of hepatitis B infection, and this mechanism may provide an unromantic form of connubial spread of infection (Blumberg *Science* **197,** 17; 1977). There is undoubtedly a need to examine closely and objectively these and other blood-sucking insects, and the entire matter deserves further epidemiological and experimental investigation. □

Multispecific antibodies

David S. Secher

THE classic work of Landsteiner (*The Specificity of Serological Reactions,* Dover, New York, revised ed., 1962) in the first half of this century demonstrated that antisera have a unique specificity. In addition to recognising and discriminating between most foreign molecules, a man (or other vertebrate) can recognise newly synthesised organic chemicals which could not have been encountered during evolution. It has often been assumed that the exquisite specificity of antisera is a reflection of a similar degree of specificity in the antibody molecules that make up that antiserum. This, of course, would require the potential production of a number of antibodies equal to the number of specificities (perhaps 10^6–10^8). An alternative explanation of the high specificity of antisera which has been favoured by a number of immunologists was stated particularly clearly by Talmage in 1959 (*Science* **129,** 1643). He suggested that each constituent antibody species in an antiserum (a normal antiserum is almost invariably a complex mixture of many different antibody species) is capable of binding to several different antigens. The pattern of such cross-reactions will be different for different antibodies and each one will be effectively 'diluted out' in the whole antiserum. The only strong binding will be to the antigen that all the antibodies can recognise, namely the original, immunising antigen. In this way a highly specific antiserum may be made up from a population of much less specific antibodies. More recently Inman has developed this hypothesis in considerable mathematical detail (in *The Immune Systems* (eds Sercarz, Williamson & Fox) Academic, New York, 1974).

Until recently there was little experimental data to favour this hypothesis and the observation of more than one antigen binding to an antibody (or to a myeloma protein) was assumed to arise from structural similarities between the two antigens (see for example Michaelides & Eisen *J. exp. Med.* **140,** 687; 1974). Over the past few years Frank Richards and his collaborators at Yale University School of Medicine have shown that the binding of two antigens (dinitrophenyl (DNP) and vitamin K$_3$) to the mouse myeloma protein MOPC 460 takes place on two spatially distinct sites in the immunoglobulin molecule. They have argued that this is therefore an example of multispecificity of the immunoglobulin and not due to a cross reaction between the antigens (Richards *et al. Science* **187,** 130; 1975). Richards *et al.* extended the observation of multispecificity to include antibodies present in immune rabbit sera and, by the elegant use of isoelectric focusing in polyacrylamide gels, showed that a second antigen could also be 'functional' in the triggering of lymphocytes to produce antibodies (see Richards *op. cit.* for review). Again the binding of other antigens to anti-DNP antibodies was studied.

How much of a contribution to the repertoire of antibody specificities could such multispecificity make? Richards *et al.* estimated that 100 haptens (antigens) might bind to a single antibody. We have used a more conservative 'degeneracy factor' of 10 in discussing antibody diversity (Secher, Adetugbo & Milstein, *Immunol. Rev.* in the press). But these numbers are still just guesses and data from other systems are clearly required to indicate how general the phenomenon is.

Cameron and Erlanger (this issue of *Nature* page 763) have now examined the binding specificities of antibodies to adenosine-5′-phosphate (AMP) (see also *Immunochemistry* **13,** 263; 1976). Three fractions of anti-AMP antibody were purified by preparative isoelectric focusing from the serum of rabbits immunised with AMP–gramicidin S. The ability of these fractions to bind

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antigens other than AMP was studied by the inhibition of enzyme immunoassays and by three other binding or cytolysis assays. In contrast to Richards *et al.* who tested more than 300 ligands to find only 2, Cameron and Erlanger appear to have found at least 2 cross-reacting antigens out of the 11 tested. Vitamin K_3 (yet again!) and caffeine were found to bind to two out of three of the antibody fractions.

Cameron and Erlanger draw an analogy between the multispecificity of antibodies and the binding to enzymes of ligands unrelated to the main substrate. Some enzymologists might disagree with interpretation of the enzyme data but in any case the analogy is not very appropriate. Enzymes are homogeneous and so multispecificity in substrate binding could not enhance their specificity in the way that Talmage, Inman and others have proposed for antisera.

There is one practical consequence of the multispecificity concept that has so far escaped comment. Monoclonal antibodies produced by the cell fusion technique (Kohler & Milstein *Nature* **256,** 495; 1975) might have unexpected cross reactivities. No such observation has been reported but it is probably too early for anyone to have systematically looked for one.

A more fundamental question that has received surprisingly little attention is the relationship between antibody affinity and multispecificity. The 'best' antibodies bind their antigens with avidities of 10^{-9}–$10^{-7}\,\mathrm{l\,mol^{-1}}$ but so far multispecificity has only been observed in the $\geqslant 10^{-6}\,\mathrm{l\,mol^{-1}}$ range. It is clear from all the published data that as one examines antigens that bind more weakly to an antibody, there is a greater degree of cross reaction with other antigens. How much biological significance do these weaker interactions have? A normal immune response develops from low affinity IgM antibodies on the surface of cells in the primary response to high affinity IgG antibodies in the secondary response. Perhaps *in vivo* there is no single 'degeneracy factor' but a spectrum ranging from multispecific, low-affinity antibodies that enable an individual with relatively few cells to recognise a maximum of antigens, to monospecific, high-affinity antibodies. The best monoclonal antibodies may thus be highly specific after all! ☐

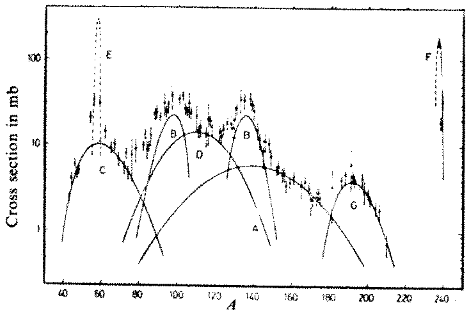
High-energy nucleus–nucleus collisions

from P. E. Hodgson

VERY many complicated processes can take place when two heavy nuclei collide at high energies, and these can be studied in a global way by measuring the mass distribution of the reaction products. A sufficient proportion of these products is radioactive, so this can be done by a radiochemical analysis of the target after irradiation. Such analyses give only the total cross sections for the production of the various fragment nuclei, and thus complement the detailed studies of individual reactions to particular final nuclear states.

Among the cross sections that can be determined by this method are those for fusion, when the two nuclei fuse together to form a compound nucleus, and quasi-fission, in which several tens of nucleons are transferred from one nucleus to another by a deep inelastic collision. Previous studies of the interaction of ^{40}Ar with ^{238}U and of ^{84}Kr with ^{238}U show that in the former case 55% of the total reaction cross section may be ascribed to fusion and 9% to quasi-fission, while in the latter there is only 4% fusion and 38% quasi-fission. These results suggest that as the mass of the projectile increases the probability of fusion falls rapidly. This is of great practical importance for those who are trying to make super-heavy nuclei by the collisions of heavy nuclei since it shows that the heavier the interacting nuclei the less likely they are to stick together, implying that heavy-ion experiments designed to produce superheavy nuclei are considerably less feasible than has been previously supposed.

As it is very important to verify this conclusion and confirm that this trend is indeed a universal one, a further measurement of the reaction products from the interaction of ^{56}Fe with ^{238}U has recently been made by Reus and collaborators from the Universities of Marburg and Manchester (*Phys. Rev. Lett.* **39,** 171; 1977). They find that 14% of the total reaction cross section is due to fusion, and 26% to quasi-fission, in line with the previous results.



Mass yield curve for the fragments from the interaction of 538 MeV ^{56}Fe with ^{238}U, analysed into components corresponding to various reaction mechanisms as described in the text.

The experiment was made by bombarding a target of ^{238}U with 538 MeV ^{56}Fe ions from the Manchester Linear Accelerator. After irradiation, the target was analysed radiochemically and the cross sections for the production of 173 nuclides were determined. The results are shown in the figure as a function of atomic mass, and show a number of distinct peaks which can be attributed to particular processes. Thus component A is assigned to fusion reactions (followed subsequently by fission) because the form of the mass yield curve in this region is consistent with that of a broad Gaussian distribution peaking at a mass value of 137±2, which is the estimated most probable mass for fusion–fission products in this interaction.

The component B has a shape and location indicating that it is due to low energy asymmetric fission following the quasi-elastic transfer of a few nucleons from one nucleus to the other. Component D is obtained by subtracting A and B from the total mass–yield curve, and its maximum corresponds to the mass number to be expected from the symmetric fission of nuclei formed by the deep inelastic transfer of several tens of nuclei between target and projectile, and so it is assigned to fission following quasi-fission, or sequential fission. Component G corresponds to those nuclei that are formed by deep inelastic transfer from target to projectile and survive sequential fission, and component C is the light quasi-fission partner. Finally the two sharp peaks at either end of the distribution, components E and F, are due to quasi-elastic transfer. They are not very well determined because many of the nuclides in these mass regions have half lives that are unsuitable for radiochemical assay. As a check on the whole analysis, the total cross section calculated for this interaction agrees with the sum of all the individual cross sections.

Integration of the cross section corresponding to each component enables the relative importance of the various processes to be found. The type of interaction that is likely to lead to the formation of superheavy nuclei is fusion of the interacting nuclei to form a compound system. This is of course expected to be rather unstable, so it soon breaks up, probably by fission. The important thing is that the compound system lives long enough for its

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properties to be studied, and this is not the case for any of the other processes identified in this analysis.

This experiment, together with the two previous ones already mentioned, gives fusion cross sections of 620, 190 and 55 mb for the interactions of Ar, Fe and Kr with uranium, showing that the higher the mass of the projectile the lower the fusion cross section. Experiments designed to produce superheavy nuclei will thus have to rely on reactions initiated by projectiles much lighter than the target, and this severely reduces the range of nuclei that might be produced. ☐

Meteorite research old and new

from Robert Hutchison

The Fortieth Annual Meeting of the Meteoritical Society was held at Cambridge, UK on 24–29 July, 1977. The successful meeting attracted a dedicated band of 'amateurs' together with astronomers, astrophysicists, chemists, geologists, mineralogists and physicists.

REPRESENTATIVE of the 'old' is the search for isotopic anomalies in meteorites and their interpretation in terms of the birth of the Solar System. This is contrasted, for me, with 'new' studies on the asteroids of today, with new experimentation on meteorites and with the discoveries of new meteorites.

Representative of classic meteorite research was the discussion over the interpretation of ^{26}Mg excesses in meteorites. Over the past few years, this ^{26}Mg excess (from decay of ^{26}Al, half life, 740,000 years) has been interpreted in two main ways. One school considers that the excess was introduced into the Solar System in pre-solar refractory grains in which ^{26}Al was already extinct (D. D. Clayton, Rice University, Houston); the other considers that ^{26}Al was present in a pre-solar nebula, condensation of which produced high temperature mineral aggregates in which ^{26}Al decayed to ^{26}Mg; in these ^{26}Mg is proportional to the ^{27}Al/^{24}Mg ratio—the isochron relationship established by G. J. Wasserburg and co-workers at Caltech.

At the meeting Clayton argued that, because stars seem to form in cold gas–dust clouds, it must be assumed that all refractory elements were condensed at planet formation in the Solar System. Ca–Al–Ti-rich inclusions in the Allende meteorite, are, therefore, condensates from an expanding supernova. This is supported by the presence of volatile-rich rims around some inclusions; at least some of the rims predate incorporation into the meteorite. This argument was taken further by Schramm, Grossman and others (University of Chicago), who now propose that the products of two supernovae were ejected into the matter from which the Solar System formed. From the earlier came the r-process nuclides (^{244}Pu and ^{129}I for example) with half lives long enough to allow survival over about 10^8 yr, until the second, smaller supernova produced, among others, ^{26}Al. Enrichment in ^{16}O was due to destruction of ^{17}O and ^{18}O in the second event. The two stage theory is based on lack of correlation between isotopic anomalies in the light elements (O, Ne and Mg) and on isotopic homogeneity in the heavy elements (Sr, U).

A probably important discovery was discussed by R. N. Clayton, who, with T. K. Mayeda (both from the University of Chicago), found that the hydrated matrix of the unusually metal-rich carbonaceous chondrite, Renazzo, has terrestrial oxygen isotope ratios: for the first time we have a potential source for our oceans and atmosphere. Of similar importance is the discovery of a strongly heated carbonaceous chondrite, 'Mulga West' from Western Australia. Few ordinary chondrites contain water, most having been heated to temperatures of about 1,000 °C; in contrast, all previously known carbonaceous chondrites have low temperature mineralogy and so are often considered as originating in comets from the outer Solar System. The findings of R. A. Binns (University of Western Australia) and Australian and British coworkers now indicate that at least one carbonaceous chondrite parent-body was heated to the same extent as the (presumably) asteroidal parents of the ordinary chondrites. The question of the sources of meteorites was addressed by C. R. Chapman (Planetary Science Institute, Tucson). Considerable spectral and dynamic data have been accumulated on the asteroids, which can now be identified as potential sources of most meteorite types. However, problems still remain in relating the relative abundances of the different meteorite types to the relative abundances which could be produced from the asteroids.

Also in the 'new' category was a search for meteorites in Antarctica by W. A. Cassidy (University of Pittsburgh), E. Olsen (Field Museum, Chicago) and K. Yanai (Japanese Institute of Polar Research). With National Science Foundation support they spent two months near McMurdo Base. Their success in finding 11 meteorites has probably sparked off a new era in meteoritics, for it showed that the finding of hundreds of meteorites by the Japanese in the Yamato Mountains was not just a flash in the pan. And meteorites found in 'blue ice' areas should be sterile, a fact noted by the organic chemist C. Ponnamperuma (University of Maryland), who did not give his paper as scheduled but was reportedly hunting meteorites in Greenland. Meteorites are normally named after places near where they are found; the lack of named geographical features in Antarctica therefore poses a serious problem in meteorite nomenclature. This was discussed by a Committee on Meteorite Nomenclature of the Meteoritical Society, whose conclusions will be communicated in due course.

Other papers of note included one by N. M. Evensen and colleagues (Lamont Observatory, New York) who confirmed earlier suspicions that rare earth elements (REE) in some chondrites are fractionated. This could be a blow to geochemists who normally use REE in chondrites as an 'unfractionated' standard for comparison with terrestrial rocks. A consortium study on the largely unshocked achondrite Serra de Magé provided a further enigma. M. Prinz and G. Harlow (American Museum of Natural History, New York) and their various collaborators showed that this stone cooled more slowly than any known basaltic meteorite; that its pyroxene minerals are similar to those in the huge, slowly cooled Stillwater intrusion of Montana. However, G. Lugmair and others (University of California, San Diego) used the Nd–Sm age determination technique to show that Serra de Magé minerals probably did not become closed systems until 4.52 Gyr ago. By that time several related meteorites had been cool, and sometimes fractured (by impact?), for over 100 Myr. This poses problems about the size and heat-source of the parent body.

The meeting did not concern itself with meteorites alone. Two sessions were on lunar studies and one covered 'Terrestial Impact, Craters and Tektites', the first three papers of which dealt with a large Russian impact structure (49°N, 59°E). It was a pity that no Russian attended the meeting, but western collaborators described this Zhamanshin structure as an impact crater some 15 km in diameter, with associated natural glasses related to tektites.

Finally, several papers relied on new,

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improved technology and experimental techniques. Data from the ion probe were presented and in one study of a fine-grained iron meteorite a scanning device on a transmission electron microscope was used to obtain high resolution spot analyses across the interface between α (low-Ni) and γ (high-Ni) metal. J. I. Goldstein and coworkers at Lehigh University, Pennsylvania intend to apply this technique to experimentally produced structures in an attempt to measure diffusion rates at low temperatures (below about 600 °C). □

More light on enzyme control

from C. J. Lamb and R. K. Dudley

It has been said in these columns that 'Plant molecular biology is not dead, but is merely awaiting the attention of competent and dedicated biochemists', (*News and Views* **254**, 13; 1975). A recent illustration of the potential contribution that biochemical studies can make to the understanding of plant biology is given by the work of Klaus Hahlbrock and colleagues at the University of Freiburg on the photocontrol of phenylalanine ammonia-lyase (PAL) levels in parsley cell cultures. By looking at the production of PAL in cell-free systems they have been able to show that the increase in PAL production observed after illumination is due to an increase in translatable mRNA and not to activation of previously synthesised enzyme.

PAL catalyses the deamination of L-phenylalanine to trans-cinnamic acid, the first step in the biosynthesis of plant compounds based on the phenylpropane unit and including lignins, flavonoids and coumarins. Its activity increases greatly following illumination of various plant tissues and the enzyme has proved popular for studying the photocontrol of gene expression in higher plants. Unfortunately, many of these investigations have relied on the effects of the antimetabolites actinomycin D and cycloheximide, both of which have many different effects in higher plants. Furthermore, density-labelling studies of the phytochrome-mediated increase in PAL activity in mustard seedlings have produced conflicting results and there is considerable controversy as to whether in this case enhancement of PAL activity is caused by activation of previously synthesised protein or by increased *de novo* synthesis. Hahlbrock's contribution has been to complement antimetabolite and *in vivo* labelling experiments with a careful study of the synthesis of PAL in cell-free systems. The results obtained to date suggest that this approach will give insight into a number of fundamental problems in plant biology.

Early work (reviewed by Hahlbrock *Physiol. Veg.* **14**, 207; 1976) demonstrated that on irradiation with blue or ultraviolet light, previously dark-grown parsley cell suspension cultures accumulate a number of flavonoids concurrent with a large increase and subsequent decrease in the extractable activity of PAL. These responses are red/far-red reversible, but neither red nor far-red light has an effect without ultraviolet preirradiation, suggesting that the initial responses are not phytochrome-mediated. The ability of light to enhance PAL activity declines progressively, and after a few hours illumination further irradiation has no stimulatory effect. *In vivo* studies with actinomycin D and cycloheximide and labelling with ^{35}S-methionine and ^{15}N-ammonia indicated that PAL was synthesised *de novo* following illumination. The light-mediated changes in the apparent rate of synthesis of the enzyme were calculated from the time-course of changes in extractable PAL activity under various programmes of illumination, and the results interpreted as reflecting short-term light-mediated increases in the concentration of specific mRNA available for translation.

This hypothesis has now been investigated directly by study of the biosynthesis of PAL in cell-free systems. *In vitro* protein synthesis with polyribosomes from parsley cell cultures was relatively low with a homologous system, but an effective heterologous system was established by supplementing the parsley ribosomes with an extract from wheat germ (Schröder & Hahlbrock *Biochim. biophys. Acta* **366**, 454; 1974). The rate of synthesis of PAL was determined by incorporation of ^{35}S-methionine into protein precipitable by a rabbit antiserum prepared against the purified enzyme (Schröder, Betz & Hahlbrock *Eur J. Biochem.* **67**, 527; 1976). Analysis of the immunoprecipitate by disc gel electrophoresis in the presence of dodecylsulphate revealed small amounts of the complete enzyme subunit (molecular weight 83,000) and relatively large amounts of shorter peptides which were also immunologically characteristic of the enzyme; an extra check by tryptic fingerprinting would have been desirable.

The capacity for PAL synthesis was much greater with polyribosomes from irradiated cells compared with those from dark-grown cells and the observed changes in the rate of PAL synthesis closely corresponded with those previously calculated from changes in the level of extractable PAL activity. The possibility that the increased capacity for PAL synthesis reflects changes in the amount or activity of ribosome-bound factors rather than changes in the level of translatable mRNA has been ruled out by extracting the RNA from parsley polyribosome preparations with chloroform/phenol and using this extract as a message in a rabbit reticulocyte lysate (Ragg, Schröder & Hahlbrock *Biochim. biophys. Acta* **474**, 226, 1977). Synthesis of PAL could only be detected using RNA from light-treated cultures. This is the first report that a mammalian cell-free system will accept a plant message for translation, and is especially interesting since PAL is an enzyme of plant secondary metabolism and is not found in animals.

These studies demonstrate an increase in the level of translatable PAL mRNA in response to illumination, but is this a result of more message being produced, or less being destroyed, or to pre-existing message being made available for translation, or indeed to a combination of these possibilities? To answer this will require purification of the PAL mRNA, a daunting task as the message is likely to be only a tiny proportion of the total RNA extracted. However, enhancement of the level of PAL activity is accompanied by concurrent increases in the subsequent enzymes of the pathway and using Hahlbrock's approach it should be possible to analyse the mechanisms whereby coordinated gene expression in higher plants is achieved in response to environmental stimuli. Of immediate interest is the phytochrome-mediated stimulation of phenylpropanoid biosynthesis in intact plants. The regulatory mechanisms may well differ from those observed with cell cultures undergoing rapid cell division and nucleic acid biosynthesis. □

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A hundred years ago

The *Frigorifique*, fitted up for the transportation of meat on the Tellier system with methylic acid, has arrived at Havre, from Brazil, with its cargo in an excellent state of preservation. It is stated that a banquet of the meat will be served during the forthcoming session of the French Association at Havre.

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review article

Why the genome does not congeal

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Since the genes in any given organism have evolved together to produce an adaptive phenotype, one might expect that rearrangement by chromosomal recombination would be maladaptive and thus wiped out by natural selection. This article reviews various theories that explain why, on the contrary, recombination is almost universal.

TEN years ago, Turner[1] asked "Why does the genome not congeal?" That is, why do organisms not evolve a single large chromosome, with no chiasmata, so that genes inherited from one parent, father or mother, are passed on without recombination to a child. This question is related, of course, to that of why organisms reproduce sexually rather than parthenogenetically[2]. The argument against recombination, crudely, is this: if an individual has survived to breed, it is likely to carry a set of genes which combine well together, and recombination will therefore have the effect of breaking up sets of co-adapted genes.

In fact, there are good theoretical reasons for expecting natural selection to reduce recombination. Thus suppose that a population has reached genetic equilibrium under selection in a uniform environment; that is, gene frequencies at all loci have reached their stationary equilibrium values under selection. Then either the population will be genetically monomorphic, in which case recombination is selectively neutral, or (as almost all natural populations are) it will be genetically polymorphic at many loci. In the latter case, selection is likely to be for reduced recombination.

Fisher[3] was the first to reach this conclusion. Serious mathematical work was started by Kimura[4], and has recently been reviewed by Lewontin[5]. The crucial question is whether there is 'linkage disequilibrium' in the population: that is, whether the alleles at different loci occur independently of one another. (Technically, suppose there are two alleles at each of two loci, say A,a and B,b. Let p_{AB} be the frequency in the population of the gamete AB, and similarly for p_{Ab}, p_{aB} and p_{ab}. Then the 'coefficient of linkage disequilibrium' $D = p_{AB}p_{ab} - p_{Ab}p_{aB}$; linkage equilibrium requires that $D = 0$.) The relevance of D is simply that genetic recombination brings a population closer to linkage equilibrium; if $D = 0$, recombination does nothing, and there is no selection for higher or lower recombination.

Selection, however, can maintain linkage disequilibrium in a population provided that (1) the loci concerned are reasonably close (that is, do not recombine too freely), and (2) there are 'epistatic' fitness interactions between them. 'Epistasis' means that the effect of substituting an allele at one locus depends on what allele is present at the other. If, for example, *AB* and *ab* were of high fitness and *Ab* and *aB* of low fitness, this would be an extreme example of epistasis. Alternatively, one could say that *AB* and *ab* were 'co-adapted' sets of genes, and that *Ab* and *aB* were not. Given such fitness interactions, it is easy to see that the frequency of *AB* and *ab* gametes will tend to be high and of *aB* and *Ab* gametes low (that is, the population will be in linkage disequilibrium), and also that there will be selection for lower recombination, because recombination will tend to produce *Ab* and *aB* gametes. In other words, either there is no selection on recombination, or selection will reduce it. This is what led Turner to ask his question.

Before seeking an answer, I first describe an experiment to measure the strength of selection. In *Drosophila*, it is possible (by using inversions and relying on the fact that there is no recombination in the male) to breed flies whose chromosomes are identical to ones present in adult wild-caught flies, and to compare their viability and fertility with flies whose chromosomes have undergone a single round of recombination since their extraction from adult wild-caught flies. Charlesworth and Charlesworth[6] performed such an experiment, and found a small (and statistically non-significant) reduction in viability, and a larger and statistically significant reduction in fertility. This measure of the 'recombinational load' confirms the theoretical prediction that selection favours a reduction in recombination. Yet recombination is almost universal. Three kinds of explanation have been suggested.

(1) Chiasmata are needed to ensure proper disjunction in meiosis, and genetic recombination is an unselected byproduct. It is hard to believe this. Male Diptera manage an effective meiosis without chiasmata. Even if this were difficult to evolve, localisation of chiasmata at the ends of chromosomes, where they would have little genetic effect, would surely be possible.

(2) Recombination accelerates evolution to meet changing conditions; species which lose or reduce recombination go extinct in competition with those which retain it. The snag with this long-term 'group selection' theory is that whenever people have looked for genetic variance in recombination rate[7-13] or chiasma frequency[14] within a species they have found it. So if there is short-term selection for reduced recombination it will be effective, whatever the long-term consequences for the species. Nevertheless, this type of explanation is assumed in many discussions of the evolution of recombination rates.

(3) There is some short-term selection for higher recombination, because real populations are not in genetic equilibrium in a uniform environment. It is with theories of this third kind that this article is concerned. They are of three kinds, and allow for the fact that the environment is unpredictable, that relatives compete with one another, and that linkage disequilibrium will arise by chance in finite populations.

An unpredictable environment

It is widely assumed that if the environment is unpredictable, so that offspring meet selective forces different from those acting on their parents, then it will pay a parent to produce recombinant gametes. I have shown[2] that this is true only if the environment is unpredictable in a special and rather implausible way. Consider an environment with a number of variable features, A_m or a_m, B_m or b_m and so on (these can be thought of as hot/cold, wet/dry and so on), and a population with alternative alleles A or a, B or b adapting individuals to different features. Then it can be shown that, even with random dispersal of offspring and changing frequencies of the alternative states between generations, any selection on recombination will favour a reduction. In particular, if alleles at two different loci, say A_1 and A_2, adapt an individual to state A_m, and alleles a_1 and a_2 to a_m, then there will be selection for reduced recombination.

Only in the very extreme case of unpredictability, in which the correlations between environmental features—say A_m with B_m and a_m with b_m—change sign between generations will there be selection for increased recombination; that is, only if hot places tend to be dry in one generation and to be wet in the next.

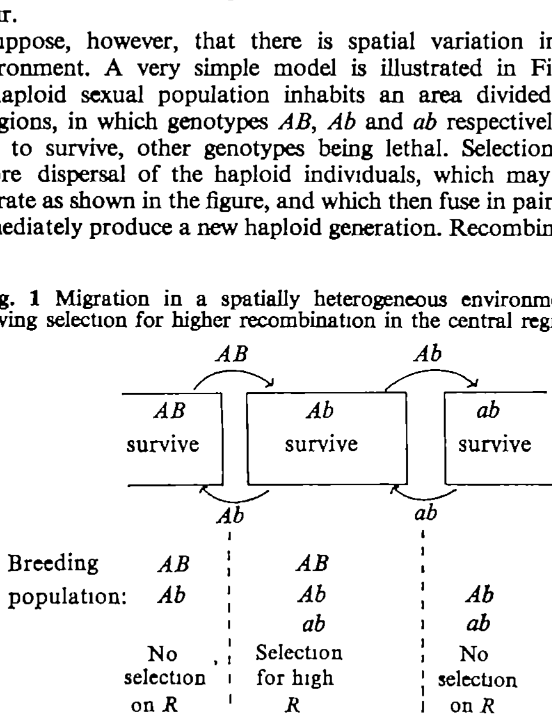
This conclusion may be counter-intuitive. If so, I think it is from anthropomorphism. We argue 'I must not put all my eggs in one basket, because I might lose them all.' But in competition between two types, say H and L for high and low recombination, what matters is not that each individual should have at least one surviving offspring, but whether individuals of type H or L have on average the larger number of surviving offspring. In these circumstances, it pays to put all your eggs in one basket if it is a better-than-average basket.

In this analysis[8], I considered frequency changes for one generation only, for a rather artificial selection regime. A more realistic genetic model was considered by Charlesworth[15], with essentially similar results. He considered a population in an environment which fluctuated either cyclically or stochastically, and found that the allele for higher recombination increased in frequency only in those simulations in which D_m (the value of D which would have been reached at equilibrium if environmental conditions were held constant) changed sign from time to time; this in effect requires that correlations between selectively relevant features should change sign, as in my model. He also found that the periodicity (or autocorrelation) of the environmental change was important.

These models of an unpredictable environment do show that there can be short-term selection for increased recombination. My own judgement of them, however, is that they show that the widespread belief that environmental unpredictability is sufficient to account for recombination is wrong, because the type of unpredictability required is of a kind which may seldom occur.

Suppose, however, that there is spatial variation in the environment. A very simple model is illustrated in Fig. 1. A haploid sexual population inhabits an area divided into 3 regions, in which genotypes AB, Ab and ab respectively are able to survive, other genotypes being lethal. Selection acts before dispersal of the haploid individuals, which may then migrate as shown in the figure, and which then fuse in pairs and immediately produce a new haploid generation. Recombination is irrelevant in the terminal regions. In the central region it is advantageous in the double heterozygotes AB/ab, since it enables them to produce viable Ab offspring.

Fig. 1 Migration in a spatially heterogeneous environment, giving selection for higher recombination in the central region.



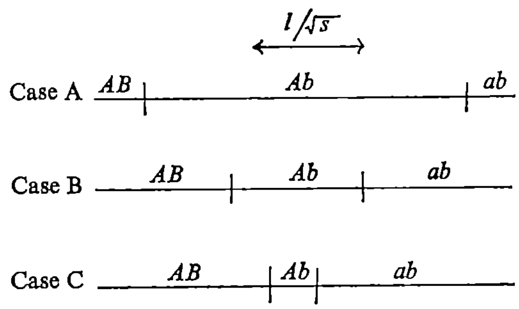


Fig. 2 Relation between the typical migration distance, $l/\sqrt{s}$, and the distance between environmental discontinuities. AB, Ab, and ab are the fittest genotypes in the respective regions.

Slatkin[16] analysed a more realistic model (Fig. 2). He considered a linear habitat with two boundaries, at one of which selection switches from favouring allele A to a, and at the other from B to b. If the boundaries are far apart (case A), two independent clines arise; there is no linkage disequilibrium and no selection on recombination. If they are coincident or close together (case C) there will be linkage disequilibrium either side of the boundaries, and selection for reduced recombination.

If the boundaries are an intermediate distance apart (of the same order as the 'characteristic distance' $l/\sqrt{s}$, where l is the typical distance an individual moves, and s the selective advantage per locus) there will be selection for higher recombination between the boundaries (case B). Hence for a spatially varying environment there can be selection for increased recombination. Unfortunately it is equally easy to think of situations (case C) in which selection favours reduced recombination.

Competition between sibs

Williams[17,18] has pointed out that if sibs compete with one another it will pay a parent to produce a genetically variable family. Computer simulation[19] shows that this can be a powerful force favouring sexual as against asexual reproduction or high as against low recombination. The basic model is shown in Figure 3. To fix ideas, imagine a population of self-incompatible hermaphrodite plants. The environment is divided into 'patches', each large enough to support one adult plant. Each patch has a number of features—5 in the simulations—each of which can be in one of two states, A_m or a_m, B_m or b_m . . . E_m or e_m. In each generation the states of each patch are randomly assigned, the 32 possible types being equally common.

Individuals are diploid, with 2 alleles at each of 5 linked loci, A or a, B or b and so on. Genotypes AA and Aa are equally adapted to A_m, and aa to a_m; the other loci are similarly related to other features. Thus an individual can be adapted to O, 1 . . . . . 5 features of the environment.

There are L patches, and hence L surviving adults. To produce a new generation, a new set of L patches is generated randomly. Each patch receives N seeds from each of R female parents. To produce N seeds, 2 parents are chosen randomly and offspring produced according to the usual laws of genetics. The 5 loci are linearly arranged, and the rate of recombination between them is determined by alleles at a sixth modifier locus.

When RN seeds have been produced in a patch, one survivor is chosen randomly from those best adapted to it. A successful parent, therefore, is one who produces many winners. Since there can be only one winner in each patch, it pays to produce as variable a family as possible. Since recombination increases within-family variability, alleles for high recombination increase in frequency.

Some results of simulating the model are given in Table 1.

Table 1 Selective advantage of recombination

Population size, *L*	Parents per patch, *R*	Offspring per parent, *N*	Total selection intensity, *NR*	Recombination fractions C⁻	Recombination fractions C⁺	Number of generations	Frequency of allele C⁺ Initial	Frequency of allele C⁺ Final	Estimated selective advantage of C⁺/C⁺
200	4	8	32	0.0	0.5	15	0.265	0.462	+0.165
200	5	10	50	0.05	0.5	10	0.262	0.387	+0.181
400	5	10	50	0.05	0.5	6	0.330	0.426	+0.182
400	5	10	50	0.05	0.25	6	0.301	0.388	+0.189
400	5	10	50	0.05	0.25	6	0.305	0.355	+0.115
Locus of C unlinked to fitness loci									
400	5	10	50	0.05	0.5	6	0.316	0.375	+0.127
400	5	10	50	0.05	0.5	6	0.272	0.325	+0.147

There is a substantial advantage for recombination. In contrast to the models discussed in the next section, the advantage does not disappear if the modifier locus is unlinked to the fitness alleles.

Thus by modifying the 'unpredictable environment' model to allow for competition between sibs, one obtains substantial short-term selection for higher recombination. There are, however, two reasons why sib competition cannot by itself explain why the genome does not congeal. First, the dispersal pattern of the species must be such that sibs compete with one another, yet meet environmental conditions different from those in which their parents survived. Second (as in the models discussed in the last section), if alleles at two loci adapt an individual to the same environmental conditions, selection will reduce recombination between them.

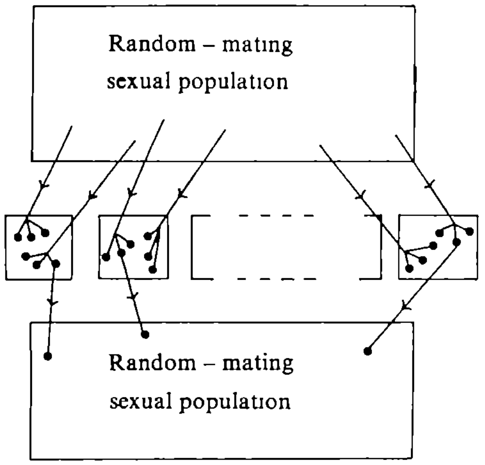
Hitch-hiking and the evolution of finite populations

'Hitch-hiking' is easiest to understand for genes which modify the rate of mutation. Suppose that a mutator gene causes a unique favourable mutation which increases in frequency and becomes fixed. If there is no recombination the mutator gene will also be carried to fixation. This theoretical possibility was discussed by Leigh[20], and Cox & Gibson[21] showed that a gene for a high mutation rate was repeatedly carried to fixation in a non-recombining population of *E. coli*.

Strobeck *et al.*[22] suggest that a similar process may select for alleles for higher recombination. Consider a particular case. A population has a balanced polymorphism for a pair of alleles *A* and *a* (for example, because *Aa* is fitter than *AA* or *aa*). A unique favourable mutation *B* occurs, say in an *AB* gamete. *B* will increase in frequency for a time, but if there is no recombination this increase will be interfered with by selection at the *A* locus. In the absence of *aB* gametes, *B* cannot go to fixation.

Suppose now that there is a pair of alleles C^+ and C^- at a third linked locus, such that recombination between *A* and *B* occurs only in C^+/C^+ genotypes. Sooner or later an aBC^+ gamete will arise by recombination, and will increase in frequency. The allele C^+ hitches a lift from the *aB* gamete it has produced, but only if it can hang on; that is, only if *C* is closely linked to *A* and *B*.

Fig. 3 Dispersal pattern for the sib-competition model, with 3 offspring per parent, and 2 parents contributing to each patch.


Random – mating sexual population
Random – mating sexual population

A simulation is shown in Figure 4. In fact the allele C^+ receives two lifts. The first can be down (case A) or up (case B), according to whether the original *B* mutation occurred in repulsion or coupling with C^+. The second, when an aBC^+ gamete is produced, is always upwards. The average effect is always to increase the allele for higher recombination.

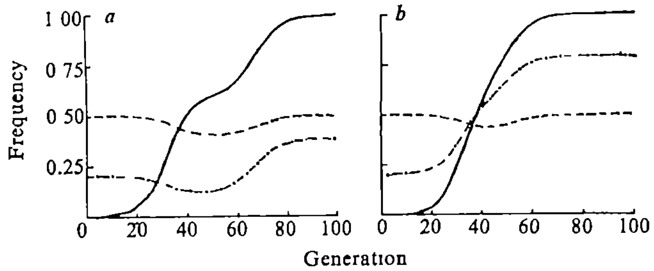


Fig. 4 The hitch-hiking effect on a gene for recombination: *a*, gene frequencies when *B* is initially in coupling with C^-; *b*, gene frequencies when *B* is initially in coupling with C^+. – – –: frequency of A; ——: frequency of B; —·—: frequency of C^+.

The process operates whenever evolutionary changes occur in a population with selectively maintained polymorphisms; to that extent it is universal. But how important is it? Strobeck *et al.*[22] simulated a range of values of selection and recombination rates. The net effect, if any, is always to increase recombination. The main limitation is that the net increase in C^+ is vanishingly small unless, in the absence of C^+, the three loci are closely linked. Hence the process explains why the genome does not congeal completely, but may not account for the high levels of recombination actually observed.

Hitch-hiking is a phenomenon of finite populations. In an infinite population *AB* and *aB* gametes would both arise by mutation. Felsenstein[23] pointed out that models showing a short-term or long-term advantage for sex are usually finite-population models. He suggested that this is because in a finite population linkage disequilibrium will arise by chance (Hill & Robertson[24]). This led Felsenstein & Yukoyama[25] to simulate finite populations initially polymorphic for alleles affecting recombination, in which a succession either of favourable or of unfavourable mutations occurred. They observed selection for higher recombination in these populations. Two favourable genes interfere with one another just as a favourable and heterotic gene interfere. A modifier which can cause recombination between two favourable or two unfavourable alleles may get a lift from the favourable recombinants it has produced.

The remaining problem

Three types of model in which selection for increased recombination occurs have been considered. Models incorporating an unpredictable environment have two drawbacks; they require an extreme and implausible type of unpredictability, and they imply selection for reduced recombination whenever several loci are concerned with the same environmental feature. Models incorporating sib competition overcome the first but not the second of these drawbacks; they introduce an additional requirement for a pattern of dispersal which, while not implausible, is certainly not universal.

Finite-population, hitch-hiking models have the required universality. They have the drawback that there is substantial selection only if the modifier locus is tightly linked to the loci affecting fitness. In effect, such models explain why the genotype does not congeal, but not why recombination is as free as it is. They will be most effective in populations which are continuously adapting to qualitatively new circumstances. Fisher[3], who, characteristically, was the first to hint at a mechanism of this kind, wrote "It is apparent that for this process to have been an effective check upon the constant tendency to increase the intensity of linkage, the stream of favourable mutants must be an abundant one."

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articles

Antarctic circumpolar current: space and time fluctuations in the Drake Passage

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New oceanic data on the structure and variability of the Antarctic Circumpolar Current (ACC) in the Drake Passage and its interaction with the atmosphere have revealed a more complex spatial structure than has been seen before. They also show a significant coherence between surface wind and deep current at short periods. These new results will be essential in understanding the role of processes in the polar regions in global climate dynamics.

OCEANIC data have been obtained from two sets of closely spaced density sections and a year-long moored current meter array carried out during two international (U.S., Argentina, Chile, U.S.S.R.) multi-ship operations in the Drake Passage (between South America and the Antarctic Peninsula) in January–March 1975 and January–March 1976. These expeditions (FDRAKE: First Dynamical Response and Kinematic Experiment) are part of the International Southern Ocean Studies (ISOS), a component of the International Decade of Ocean Exploration of the Intergovernmental Oceanographic Commission of UNESCO. The atmospheric data are from an analysis of surface pressure for 1975 provided by the Australian Bureau of Meteorology in Melbourne. The data will be discussed in greater detail in several papers to be published elsewhere[1–4] but we present here a summary of the major results on the structure of the flow field and its response to atmospheric and bottom topography interactions.

The primary purpose of FDRAKE 1975–76 was to begin to establish a 'climatology' of the ACC system in the Drake Passage by the use of monitoring sections of current meter arrays and closely spaced hydrographic measurements. The mooring arrays were deployed and recovered by the current meter group from Oregon State University; and the hydrographic and chemistry work was carried out by groups from Texas A & M University and Oregon State University with technical support from the Scripps Institution of Oceanography. The hydrographic work in 1975 (refs 5–7) and 1976 (refs 8–10) included measurements of temperature, salinity, oxygen, phosphate, silicate, nitrate, and nitrite. When combined, these data provide a sharper picture of water masses and their characteristics in the regions adjacent to the Drake Passage, and they facilitate interpretation of data from the moored arrays. Data from the cruises were combined to give a nearly synoptic picture of the polar front zone from the Drake Passage to the Falkland Plateau and information on a second frontal zone, the Weddell–Scotia confluence. The work on the polar front zone is discussed in detail elsewhere[2,3].

A banded spatial structure is unambiguously revealed by the closely spaced hydrographic measurements[4]. In 1975, the stations occupied by the RV Melville were spaced 50 km apart, and in 1976, the station spacing was 45 km, in contrast to the previous work which had spacings generally greater than 100 km. This increased resolution has revealed vertically coherent bands of isopycnal slope steeper than the average downward slope of isopycnals towards the north, so that the density field has a step-like appearance.

The general characteristics of all the closely spaced sections are similar. These density steps produced cores of high geostrophic

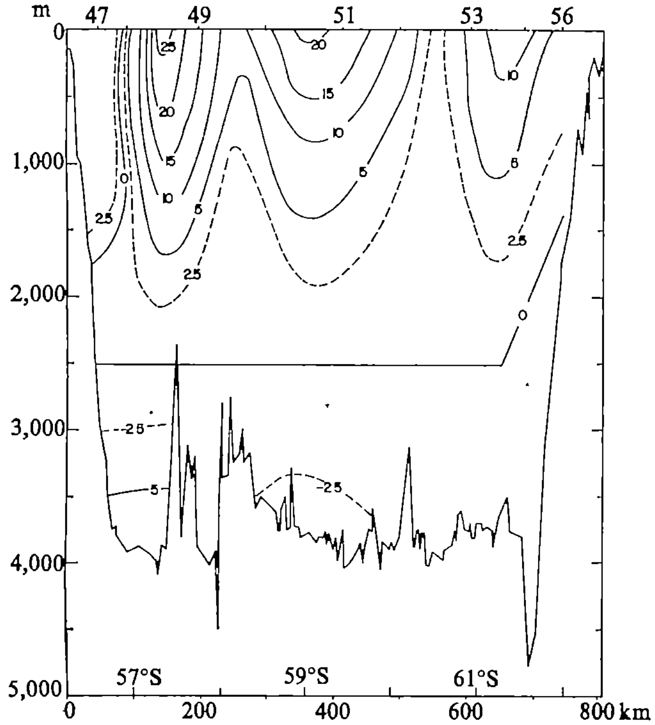

Fig. 1 Geostrophic velocity perpendicular to a line (running 332 °T) across the Drake Passage (positive eastward). The flow is referenced to 2,500 dbar; south of 61 °S, the greatest common sample depth was used as reference for station pairs which did not reach 2,500 dbar

velocity shear. In 1975, the central Passage showed bands of eastward geostrophic speeds ranging from 25–30 cm s^{-1} separated by regions of lower speed (0–5 cm s^{-1}) or slight westward flow (Fig. 1). This core-like structure was observed again in March 1976 when a line of hydrographic stations was made from the RV Thompson approximately 25 km to the east of the current meter array. Three bands of large horizontal density gradient, or cores of relatively strong flow, were observed in this section. The detailed structure of these cores will be discussed elsewhere[4].

Data from cruises of the Ob in 1958 (ref 11), the Washington in 1969 (ref. 12) and the Hudson in 1970 (ref. 13) also provide some evidence for the bands of relatively high horizontal density gradients embedded in the current in Drake Passage. However, the station spacing on these cruises was too large to permit unambiguous description of the detailed current structure. Callahan[14] has observed a similar structure, but with larger scales, in the ACC region south of Australia.

The characteristic scales of the high speed cores is the order of magnitude (20 km) to be expected if the fluid responds (to atmospheric input, bottom topography interaction and/or instabilities) on the scale of the Rossby radius of deformation (defined as $[(g/\rho)(\mathrm{d}\rho/\mathrm{d}z)]^{1/2}H/(2\Omega\ \sin\theta)$ where g is gravity, ρ density, H a characteristic depth (here chosen to be 1,000 m), Ω the Earth's rotation rate, and θ the latitude) averaged over the first 1,000 m. This is the first observation of fluid structure on this scale in this region, although mesoscale structure has been observed and suggested theoretically in other oceans[15]. J. S. Allen and M. McCartney (unpublished data) have shown that stratified, time-dependent flow through Drake Passage topography can lead to a core-like structure, we hope that these new data will stimulate further theoretical work on ocean response to these interactions in the southern ocean.

In February 1975, an array of 42 current meters on 15 moorings was deployed in the Drake Passage: 23 were recovered after three weeks, and 19 were recovered during February 1976, having measured currents over periods from 220 to 350 d.

These long-period records give us a unique data set for this region to define temporal and spatial statistics (previous measurement periods were a maximum of 13 d). To estimate time scales, an autocorrelation function for velocities in the through-passage direction (062 °T) is calculated[1] for the long-term current record nearest 2,700 m. The period of the first zero-crossing of the autocorrelation function, which averaged 14 d, is an estimate of the time-scale of dominant energy containing motions. The period of independence of the records is estimated at 7 d. The 7-d averages of flow in the through-passage direction vary from −2.9 (westward) to +7.6 (eastward) cm s^{-1} over the measurement period of 1 yr Only two of these mean flows are significantly non-zero at a 95% confidence level. Figure 2 shows the variation in the 14-d averaged through-channel flow over the year. A three regime structure is marginally present, reminiscent of the three-core hydrographic data. Note the strong changes on the northern side of the Passage The low frequency variability is strongest on the northern side of the Passage, while the high frequency variability does not seem to change significantly over the width of the Passage

The detailed statistics of this long-term variability of the deep flow will be discussed elsewhere[1]. It is, however, interesting that the observed large variability in the flow at 2,750 m now seems to be one of the main reasons for the large range of transports estimated by various authors[12,13] since in making these estimates short-term averages of deep currents were used to reference the baroclinic transports

To estimate space scales, we note that the Rossby radius relevant to deep flow near the mooring number 10 (59°8′S, 63°4′W) is about 20 km. Thus, it is plausible that correlations of horizontal velocity at distances greater than about 40 km should be small[15] This expectation is borne out by the statistical analysis of the current measurements[1] which show no significant correlations over the record lengths at the mooring spacing of about 75 km. The effect of this observed spatial variability on the total transport is discussed elsewhere[1,4]. Significant coherences between moorings have been found for some events shorter than the record length, but

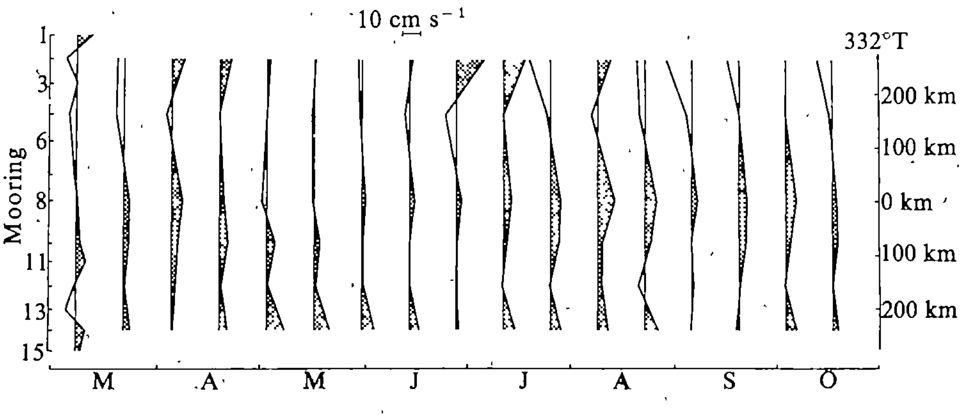

Fig. 2 14-d Averages of flow at 2,500 m from the current meter data, March–October 1975 The coordinate system is oriented in the cross- (332 °T) and through- (062 °T) passage directions. The shaded regions represent positive through-passage flow. During March a larger number of short-term moorings were deployed

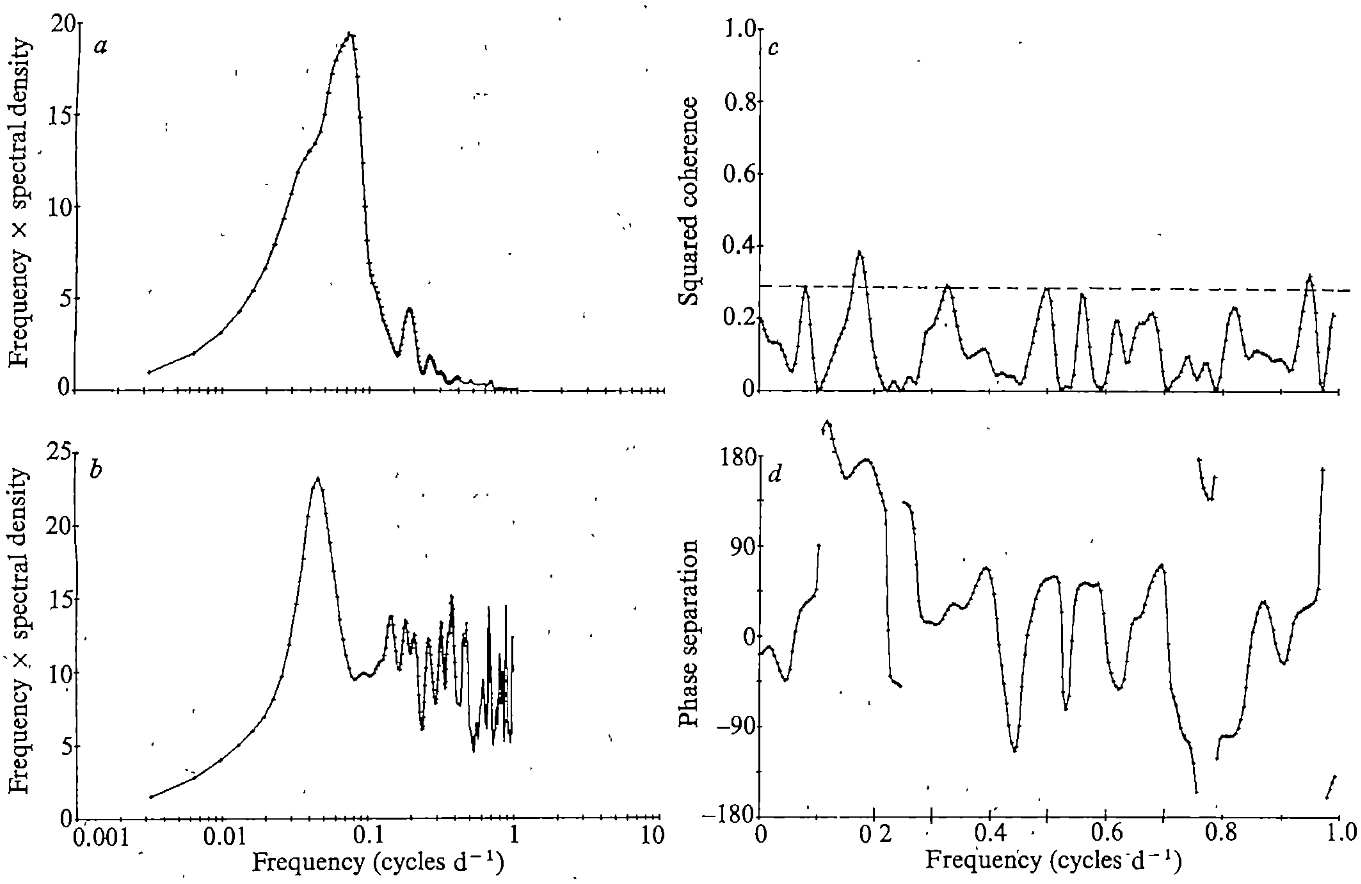

Fig. 3 *a* Spectrum of currents (at mooring 10. 1,519 m, 59°8'S, 63°4'W); *b* spectrum of geostrophic wind (components in the 062 °T, through-passage direction) in the Drake Passage (units are $cm^2\ s^{-2}$ cycles d^{-1} per unit bandwidth (currents); $m^2\ s^{-2}$ cycles d^{-1} per unit bandwidth (wind), *c*, squared coherence of currents and wind, and *d*, phase of currents and wind. Time series are 308 d long, beginning 27 February 1975 See text for discussion of coherence near the 5.5-d scale 95% confidence limits are indicated

these are still preliminary studies. The current meter array in place during 1976 and that designed for 1977 have separations of smaller scales in order to examine velocity correlations more closely.

An analysis of the surface meteorological data has also been carried out for the Drake Passage; 12-h values of pressure and pressure differences have been extracted from the 12 months of 1975 surface pressure analysis. Ten values were used over an area 1,000 × 1,000 km centred on the moored array. Both the pressure spectrum and the pressure difference spectrum, interpreted as geostrophic wind, show a number of peaks in the range of periods from 2 to 14 d, with the bulk of the energy falling at long periods (greater than 14 d). The current meter spectra show peaks near 4 d and 6 d with the bulk of the energy falling between 10 and 100 d (see Fig. 3). Coherence calculations between the through-passage current at 1,019 m and 1,519 m on mooring 10 and the through-passage wind show a significant (at the 95% significance level) peak in the coherence squared at a period of 5.7 d with a stable phase over the peak of about 172°. This coherence is good evidence that atmospheric effects are transmitted deep into the ocean at these high latitudes. It is, of course, possible that this is not a local effect. Since the atmospheric spectra show similar features to the west of the Drake Passage, we may be seeing here an oceanic response which is representative of a larger-scale air–sea interaction in the South Pacific Ocean. The currents are nearly out-of-phase with the local wind, suggesting that a larger-scale interaction may be at work.

The observed coherence between deep ocean currents and wind agrees with calculations by Philander[16] which show that at frequencies less than inertial, and for forcing at large horizontal scales, there can be a response even in the deep water. Philander notes that the large eastward travelling cyclones that dominate the spectrum of surface winds in mid- and high-latitudes should force a response down to the ocean floor in such a weakly stratified region, and that measurements between Iceland and Scotland[17] have shown a correlation between surface atmospheric pressure and deep ocean currents at depths from 300 to 1,000 m. By his calculation, a similar response should be seen in the Drake Passage (the stratification is weak enough and the storm systems are large enough). Therefore, he suggests that the observed variability of deep currents in the Drake Passage is correlated with atmospheric storms; the data tend to bear out this suggestion.

Another question of some interest for Southern Hemisphere air–sea interaction is the existence of semi-annual wave components in the current meter data, which can be compared with the well known semi-annual atmospheric oscillations[18]. Surface ocean data, from sea level and ship drift observations, have shown such a variation[19]. A wave fit to the data from all the FDRAKE moorings shows significant amplitude (defined as greater than twice the standard deviation) at this period at all depths. The magnitudes of the semi-annual component range from 5.7 cm s^{-1} at a depth of 1,019 m to 0.9 to 1.3 cm s^{-1} at a depth of 2,750 m for the through-passage component. The cross-passage component also shows a significant amplitude, with a maximum at the shallower depth. This data set is the first which is long enough to show evidence of such long-term response in the deep water. The possibility of such deep forcing by wind adds a new facet to the complexity of air–sea interaction in high latitudes.

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New Miocene locality in Turkey with evidence on the origin of *Ramapithecus* and *Sivapithecus*

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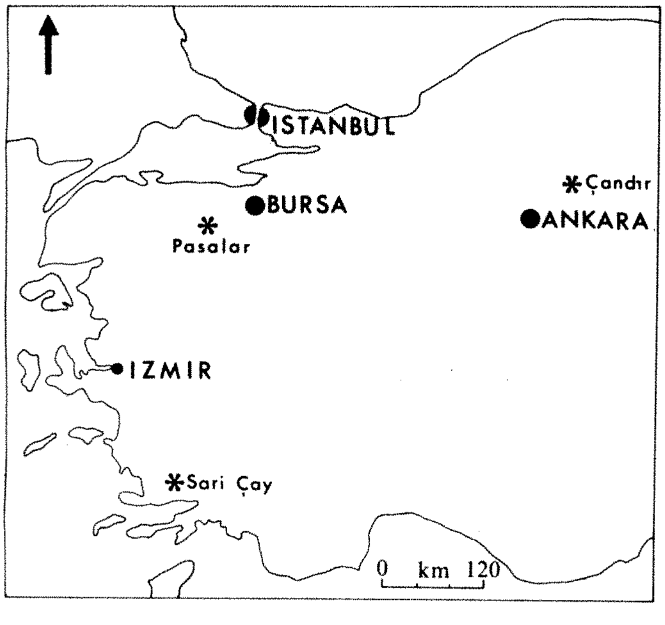
Collections in early Middle Miocene deposits at Pasalar in Turkey have yielded a very rich fauna. Included in this are two hominoid species referred here to Sivapithecus darwini *(Abel) 1902 and* Ramapithecus wickeri *(Leakey) 1962. These are both more primitive morphologically and earlier in time than other species of these genera, and they provide evidence that* Sivapithecus *and* Ramapithecus *are closely related and that their early diversification may have occurred not in Africa but in Eurasia.*

THE mammal-bearing locality at Pasalar, Turkey, is situated 68 km south-west of Bursa, 12 km south-west of Kemalpasa, and about 6.5 km south of the main Bursa–Susurluk road, NW-Anatolia (Fig. 1)[1]. The site was discovered in 1969 during a lignite exploration[2] in the Neogene basins of Anatolia, and is situated near the Southern edge of the Neogene Gönen Basin (west of Bursa)[3]. The lignite survey was inaugurated by the Turkish government, and reconaissance trips and field work were done in 1965–1969 by a group of geologists from the Bundesanstalt für Geologie und Rohstoffe (Hannover, FRG) in collaboration with the Maden Tetkik ve Arama Enstitüsü (MTA, Ankara)[2]. During the field work about 80 fossil vertebrate localities of late Neogene and early Pleistocene age were discovered. Some of these localities were subsequently investigated by a German research team under the leadership of the late Professor Sickenberg in collaboration with Turkish colleagues of the MTA and with the financial support of the Deutsche Forschungsgemeinschaft[2].

Fig. 1 Map of northwestern Anatolia showing the positions of Pasalar and Candir.



Stratigraphy

The fossilferous deposits at Pasalar are exposed in a small road cutting. They are resting directly on Palaeozoic granitic rocks which are clearly exposed on the west side of the road. The fossiliferous sediments consists of 2.2 m of silts and sandstones, often quite coarse, with a considerable thickness of apparently unfossiliferous silts and fine sandstones further up the section to the north and east.

The section exposed on the eastern wall of the roadcut, about 750 m south of the village of Pasalar, is as follows:

0.8 m grey-green silt, unbedded, with many small calcareous concretions, some rare irregularly distributed quartz grains, and many chips of bone of larger mammals and rare isolated teeth of micromammals.
1.0 m whitish to light grey sand, fine grained, unbedded, with frequent lenses of coarser sands and gravels (mainly quartz pebbles up to several centimeters in diameter), unbedded and badly sorted, non calcified. Abundant fossils.
0.4 m grey-green silt, with occasional small red spots, many cm-sized white calcareous nodules, unbedded, unfossiliferous.
Unconformity
Palaeozoic granitic rocks and undifferentiated metamorphosed rocks.

The relatively coarse sediments that make up the fossiliferous series are overlain by a finer grained more strongly bedded succession. Discontinuous beds and lenses of coarse sands, which decrease in frequency up the section, occur throughout. The depositional environment was fluviatile, with rapid sedimentation in high energy conditions in the main fossiliferous bed followed by quieter conditions, but with occasional high energy episodes, later on. This accords well with the location of the sediments, situated as they are on the southern border of the Neogene Gönen Basin at the base of the sedimentary sequence. They probably represent initial molasse-like fill of a tectonic basin near the lower slopes of a mountain range[4].

Table 1 Pasalar faunal list

Sivapithecus darwini (this work)
Ramapithecus wickeri (this work)
Soricidae sp. (small form)
Talpidae sp. 1
Galerix cf. *moedlingensis*
Erinaceinae sp.
Chiroptera (middle sized form)
Prolagus cf. *oeningensis*
Alloptox n.sp. 1
Cricetodon (*'Palaeocricetus'*) sp.
Megacricetodon sp.
Democricetodon sp.
Sciuropteridae sp. indet.
cf. *Spermophilinus bredai*
Pseudodryomys sp.
cf. *Rhizomys* sp.
Ctenodactylidae sp. indet.
Dipodidae sp. indet.
Amphicyon cf. *major*
Hemicyon sansaniensis
Plithocyon sp.
Ursavus cf. *primaevus*
Ursavus aff. *intermedius*
Plesiogulo n. sp.
Proputorius sp.
Trochictis sp.
Lutrinae sp. I
Protictitherium intermedium
Protictitherium aff. *gaillardi*
Protictitherium sp. I
Percrocuta (*Percrocuta*) *miocenica*
Percrocuta sp. I
Pseudailurus cr. *quadridentatus*
Pseudailurus (*Schizailurus*) *larteti*
Orycteropus sp.
Deinotherium giganteum
Gomphotherium angustidens
Anchitherium sp.
Tapirus sp.
Beliajevina tekkayai
Listriodon splendens
Schizochoerus sp.
Conohyus sp.
Cervidae indet.
Protoryx cf. *carolinae*
Prostrepsiceros cf. *rotundicornis*
Bovidae indet.

Fauna

The bulk of the fossil remains come from the sand and gravel layer. Within that bed the fossils are concentrated in the unconsolidated gravels and consist largely of isolated teeth of mammals ranging in size from insectivores to elephants. There are also many fragmentary postcranial elements, and these occur, together with the isolated teeth, in very great local concentrations.

The faunal list (Table 1) for the main fossiliferous bed is provisional except for the carnivores[5], proboscidians[6] and rhinoceroses[7]. The description of the other groups is under preparation, and a preliminary account of the primates is given here. They are referred to two species of *Ramapithecus*[8-10] and *Sivapithecus*[11-13] on the basis of morphological similarities with previously described species.

In the lithostratigraphic subdivision of the late Neogene in Western Anatolia, the Pasalar sequence represents an equivalent of the Middle Miocene Turgut member. More specifically its mammalian fauna can be correlated with the fauna from Prebreza (Yugoslavia)[5], and it is somewhat earlier than the faunas of Bjelometscheskaya (Northern Caucasia) and Sansan (France)[1]. It is also one of the earliest of the Anatolian Miocene *Anchitherium* faunas[1]. In the traditional European timescale Pasalar corresponds with the early Vindobonian[5], slightly older than Sansan. In the biozonation of the Mediterranean Neogene based on mammals it belongs to the NM zone 5 (ref. 14). In the sequence of European continental mammal ages/stages, proposed by the Symposium München 1975, the NM zone 5 is the uppermost member of the Orleanian age/stage[15]. In terms of the marine stratigraphy of the Paratethys and Tethys the Pasalar niveau would probably correspond to the Tharkanian (Eastern Paratethys), Lower Badenian (Central Paratethys) and Upper Langhian (Tethys) respectively. The radiometric age of the Langhian timespan is between 15.03 and 16.27 Myr (ref. 16).

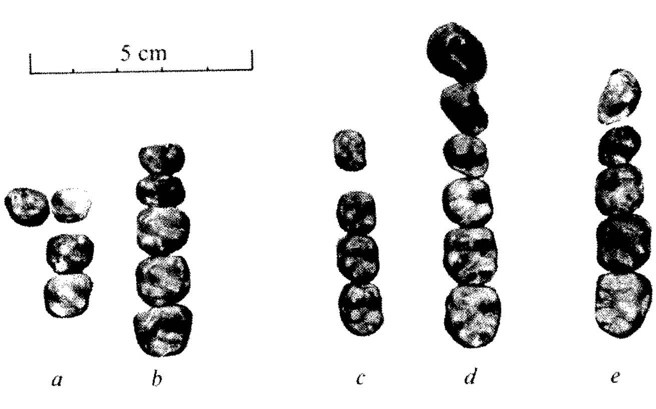
Fossil primates

The collection of primates from Pasalar consists of 100 isolated teeth : 14 of these are broken, allowing measurement of enamel thickness, but the rest are intact and well preserved. They group readily into two size categories, large and small, and on this basis a minimum number of individuals of 20 has been calculated, taking account also of the degree of wear of the teeth and whether they are from the left or right side, 12 for the large category based on 69 specimens, and 8 for the small category based on 31 specimens. Most of these are from the main fossiliferous bed, but three specimens are known from the overlying grey–green silt.

There are 11 incisors and canines in the collection. Five of these are upper central incisors, the large specimens having an expanded lingual pillar, so that they are intermediate in morphology between *Proconsul nyanzae* and *Sivapithecus indicus*, while the small incisors are similar in morphology to *Proconsul* incisors. In size the large specimens are equivalent to the I^1 of *Proconsul major* while the small ones are equivalent to very small *P. nyanzae*. (*Proconsul* here is considered to be a valid genus[10].) The five canines are all from the left side and are very similar to each other both in size and morphology (Fig. 2*d*). They are the same size as *Sivapithecus indicus* canines, and it is assumed that they belong to the large size category.

There are 22 premolars in the Pasalar collection. The P^3 is similar in size to P^4 (Fig. 2*b*) and is not buccally elongated. The P^4 is represented by eight specimens in the large category, all from the left side, and one specimen in the small size category. They are similar in morphology to *Sivapithecus* and *Ramapithecus* except that they are slightly broader relative to length. The P_3 samples are much more distinctive. The crowns are robust and low crowned and they have prominent mesiolingual cingula which end consistently in small-to-large beak-like processes (Fig. 2*d*, *e*). These are not usually seen in *Sivapithecus* species, although the size and degree of robustness of the crown is similar to

Fig. 2 Composite tooth rows of Pasalar primates. *a*, Left and right dp^4 and left M^1–M^2 of *Ramapithecus wickeri*. *b*, Left P^3–M^3 of *Sivapithecus darwini*. *c*, Left dp_4 and M_1–M_3 of *Ramapithecus wickeri*. *d*, Left C–M_3 of *Sivapithecus darwini*. *e*, Right P_3–M_3 of *Sivapithecus darwini*.



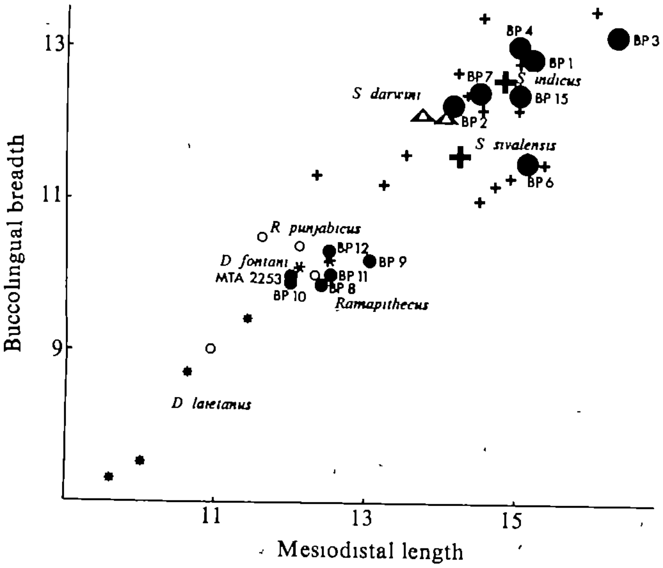


Fig. 3 Bivariate plot of M_3. Black circles indicate the Pasalar specimens, the smaller ones representing the small specimens and the large ones the large specimens. BP, Bursa-Pasalar; MTA, Maden Tetkik ve Arama Enstitusü, Ankara. Triangles indicate the type specimens of *Sivapithecus darwini*. The + signs indicate the Siwalik sample of *Sivapithecus* with the means for *S. indicus* and *S. sivalensis* taken from ref. 13 shown enlarged. The very small open circles represent *Ramapithecus punjabicus*. The stars represent small samples of *Dryopithecus laietanus* and *D. fontani*.

that of *Sivapithecus indicus*. The P_4 crowns are much more similar to those of *Proconsul* species, particularly their constricted trigonid regions and the presence of buccal cingula.

There are 18 upper and 32 lower molars from Pasalar. The size ranges in all the teeth are approximately equivalent to the ranges of *Sivapithecus indicus* for the large size category, and *Ramapithecus* species for the small category (Fig. 3). The upper molars are extremely similar in morphology to each other and to the molars of *Sivapithecus indicus*, and are quite unlike the molars of *Proconsul* species (Fig. 2*a*, *b*). This is mainly due to the presence on both of thick crown enamel which results in low rounded cusps, constricted trigon basins, and blurred occlusal ridges. Enamel thickness on the molars varies from 1.7–2.5mm in unworn teeth of the large size category to 1.1–1.5mm on slightly worn teeth of the small size category. The lower molars also have low rounded cusps and constricted talonid basins due to greater enamel thickness, and in this they resemble *Sivapithecus* and *Ramapithecus*, but in general proportions the lower molars are more like those of *Proconsul*. This is partly due to the buccal cingulum, which is prominent (Fig. 2*c*, *e*) and imparts a squared outline to the crown of M_1 and M_2, but also the trigonid is constricted and the M_3 has an elongated talonid narrowing distally. This *Proconsul*-like morphology of the lower molars contrasts with the *Sivapithecus*-like morphology of the upper molars, but there is little reason to doubt that the uppers and lowers at Pasalar are associated. Finally there are four deciduous teeth in the collection from Pasalar (Fig. 2*a*, *c*) all belonging to the small-size category.

Phylogenetic implications of fossil primates

The Pasalar primate sample of hominoid primates has been divided into two size classes (Fig. 3). They are too distinct to be the result of sexual dimorphism within a single species, but apart from that they are very similar to each other morphologically. There are, however, some morphological differences between the two groups, particularly in the I^1 and P_3, and we conclude, therefore, that they represent two different but closely related taxa. Both taxa are broadly intermediate in morphology between early Miocene *Proconsul* species and later Miocene *Sivapithecus* and *Ramapithecus* species, but they must be classified with the latter rather than the former because they share apomorphus (derived) characters with the later species of *Sivapithecus* and *Ramapithecus*, for example the thick enamel on the molars. The characters they share with the early Miocene species, such as molar cingula and narrow trigonids, are either primitive characters retained by both groups or synapomorphies present in all the dryopithecines.

The large species is the same size as *Sivapithecus indicus* from the Siwaliks[12]. But, it differs from *S. indicus* in many respects, particularly in the morphology of the central incisor, third premolar and lower molars, as well as being rather earlier in time. It also differs from *S. sivalensis* in many of the same ways, and most resembles *Sivapithecus darwini* (Abel)[11,12]. This is a poorly represented fossil species known from deposits in the Vienna basin that are about the same age as the Pasalar deposits. The morphology and size of the primate specimens from the two localities are almost identical, and the Pasalar species is therefore referred to *Sivapithecus darwini* until further discoveries, especially of mandibular or maxillary material, make a more detailed assessment possible.

The teeth representing the small species from Pasalar are very similar to those of the mandible recently described from Candir[5], Turkey. This has been referred to *Ramapithecus wickeri*[9] on the basis of mandibular as well as dental morphology. The Candir deposits are similar in age to those of Fort Ternan, which is the type locality of *R. wickeri*, but the Pasalar deposits seem to be a little older, and related to this the Pasalar *Ramapithecus* is slightly more primitive than *R. wickeri* from Fort Ternan and Candir. On present evidence the difference is not considered great enough to justify a new species name for the Pasalar form, so it will be retained in *R. wickeri*.

Stratigraphically the Pasalar deposits are intermediate in age between the early Miocene deposits of East Africa and the later Miocene dryopithecine localities of Europe and Asia[13]. This raises the possibility that the primates from Pasalar, which have also been shown to be intermediate morphologically, may provide a phylogenetic link between early Miocene *Proconsul* of Africa and later Miocene *Sivapithecus* and *Ramapithecus* of Eurasia. In this event, the great degree of similarity exhibited by *S. darwini* and *R. wickeri* from Pasalar, which suggests a closer relationship between *Ramapithecus* and *Sivapithecus* than hitherto recognised[10,13], indicates that there may be represented at Pasalar a very early stage in the origin and radiation of these two genera. This radiation was almost entirely confined to Europe and Asia, with the exception of *R. wickeri* from Fort Ternan (and some doubtfully assigned specimens of *Sivapithecus*), and although the origin of this radiation will probably ultimately be traced to Africa, on present evidence it seems that its initial diversification took place outside of Africa and probably in Central Asia.

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A genetic model for sympatric speciation through habitat diversification and seasonal isolation

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Based on studies with natural populations, we offer a genetic model for sympatric speciation that could have broad application to polyphagous animals with habitat differences. The model comprises three steps, each of which includes simple genetic changes and plausible selective forces.

ALLOPATRIC speciation as the almost universal, single mode of speciation in bisexual animals has been the subject of lively dispute[1–6]. As an alternative, Maynard Smith[7] proposed a theoretical model for sympatric speciation through disruptive selection in a two-niche situation. The details of this model were subsequently extended, especially for plants[8].

Until now, the most convincing evidence supporting the application of Maynard Smith's model to bisexual animals comes from studies with monophagous insects that form divergent host races[9–12]. In fact, Bush[5,13] suggested that sympatric speciation through disruptive selection is restricted to host specific, phytophagous or parasitic animals. Far more controversial are cases of proposed sympatric speciation through, for example, diversification in habitat association or seasonal distribution[1,3,14–16]. Unlike the cases involving host races[9], there are no step-by-step genetic models for this type of speciation mainly because there is a lack of information on the inheritance of ecophysiological response patterns controlling habitat preference and seasonality in animals.

Work with two green lacewings, *Chrysopa carnea* Stephens and *Chrysopa downesi* Banks (Insecta: Neuroptera), has shown that single allele differences at two loci result in gross alterations in photoperiodic responses controlling seasonal timing of reproduction[17]. Also, allelic changes at three loci can explain the sympatric speciation of *C. downesi* from a *C. carnea*-like ancestor[18]. Based on these and other analyses of genetic differences between *C. carnea* and *C. downesi*, we present a genetic model for sympatric speciation through habitat diversification and seasonal isolation. This model involves three steps—each of which includes simple genetic changes and plausible forces of disruptive selection. We propose that our specific model has broad application to animals that differ from sympatric relatives in both habitat association and in reproductive season. We also show that Maynard Smith's general model[7] for sympatric speciation is applicable to bisexual, polyphagous, as well as monophagous, animals.

Reproductive barriers

C. carnea and *C. downesi* readily hybridise in the laboratory and produce fully viable and fertile F_1 and F_2, as well as backcross offspring; in these conditions, there are no behavioural or post-mating reproductive barriers between the two species. But, no hybrids are found in nature and the two species are kept clearly separate through the action of two types of premating reproductive barriers—differences in their habitats and differences in their seasonal periods of reproduction[19].

Reproductively active *C. carnea* adults, which are light green, occur in grasslands and meadows, in cultivated fields, occasionally on deciduous trees, and rarely on conifers. In these habitats, the adult coloration provides camouflage, presumably against vertebrate predators. At the end of summer, as the adults enter reproductive diapause, they move to the senescent foliage or leaf litter of deciduous trees where they overwinter[20,21]. This movement is accompanied by a colour change (from light green to reddish brown) that maintains the camouflage in the overwintering site. In contrast, *C. downesi* adults do not change colour and the species is restricted to coniferous trees, where the very dark green colour of the adults provides camouflage. *C. carnea's* occasional occurrence on coniferous trees indicates a slight overlap in habitat with *C. downesi*; but, in general, the two species are spatially separated.

Adults of both species undergo diapause, during which reproductive behaviour, ovarian development, and sperm transfer are suppressed. *C. carnea* enters diapause at the end of August and it remains in diapause until mid-January or February[22]. Postdiapause mating takes place before the vernal equinox, and the first postdiapause generation adults emerge about June, after which two more summer generations are produced. Therefore, mating by *C. carnea* occurs during late winter and also during summer[23]. In contrast, *C. downesi* has only one generation per year, and the adults, on emergence in June, enter a diapause that persists through summer, autumn and winter[18]. This species ends its diapause around the time of the vernal equinox[24]; mating takes place in early spring. Therefore, *C. downesi* is reproductively inactive during *C. carnea's* entire reproductive period.

The seasonal cycles of *C. carnea* and *C. downesi* are primarily controlled by photoperiod[25]. Their asynchrony largely results from *C. downesi's* short day–long day requirements for diapause prevention, and for diapause termination. These short day–long day requirements evolved from photoperiodic response patterns that *C. carnea* and *C. downesi* share, that is, responsiveness to a change in daylength and responsiveness to the actual duration of daylight (or darkness) per 24-h period[17,25,26].

The species-specific responses are under genetic control, and a pair of alleles at each of two loci regulate the expression of the *C. carnea* versus the *C. downesi* response pattern[17]. Homozygous recessive alleles at both loci result in the short day–long day requirements that underlie the univoltinism of *C. downesi*.

The preoviposition period of non-diapausing *C. downesi* is considerably longer than that of non-diapausing *C. carnea* (Table 1). The average preoviposition period of F_1 and F_2 hybrids and backcross progeny, reared in diapause-averting conditions is also consistently longer than that of *C. carnea*, and it is usually intermediate to those of *C. carnea* and *C. downesi* (Table 1). The range in the preoviposition period in the F_2 intercross progeny (see ranges on Table 1) shows very little overlap with the *C. carnea* range. This suggests that even one *C. downesi* allele at one of the two diapause-controlling loci can lengthen the preoviposition period. In addition, modifier genes could also be involved.

Genetics of adult coloration

To determine the mode of inheritance of the dark green adult coloration of *C. downesi*, we made reciprocal P_1 and F_1 crosses between *C. carnea* and *C. downesi*. We scored the colour of the F_1 and F_2 offspring using three broad categories: 'true *carnea*', 'true *downesi*', and 'intermediate'. We also divided the 'intermediate' category into three subcategories: '*carnea*-like', 'true intermediate', and '*downesi*-like'.

All the F_1 offspring of the two reciprocal crosses of *C. carnea* × *C. downesi* fell into the 'intermediate' category; they ranged from '*carnea*-like' to '*downesi*-like' intermediates. In the F_2 progeny, coloration occurred in the three broad categories in ratios that do not differ from those expected for the segregation of a single pair of autosomal alleles—one recessive, one semidominant (Table 2). The 'intermediate' individuals again varied from '*carnea*-like' to '*downesi*-like'. Therefore, we conclude that *C. downesi* coloration is due primarily to a single, semidominant autosomal allele (which we designate as *G*); expressivity of the dark green colour is apparently modified by polygenes.

Sympatric speciation of *C. downesi*

On the basis of the distributional and biological[25] characteristics of *C. carnea* and *C. downesi*, we conclude that *C. downesi* is the more recently evolved. The multivoltine *C. carnea* occurs over most of the Holarctic Region, whereas the univoltine *C. downesi* is restricted to the more northern regions of North America. We know of no place where *C. downesi* is present without *C. carnea*, nor are we aware of evidence that the two species were ever geographically isolated.

Table 1 Preoviposition period of conspecific and interspecific crosses between *C. carnea* and *C. downesi*

Cross* ♀ ♂	Preoviposition period (d) x±s.d. (no. of pairs)	Range
Intercross		
P_1		
d^1 × d	8.5±1.9 (6)	7–12
d × c	10.5±4.5 (6)	7–19
c × d	12.6±2.8 (5)	10–16
c × c	5.4±0.6 (5)	5– 6
F_1		
dd × dd	8.2±0.97 (9)	7–10
dc × dc	5.3±0.63 (13)	4–6
cd × cd	5.9±0.88 (10)	5–7
cc × cc	4.3±1.07 (10)	4–6
F_2		
dd-dd × dd-dd	13.0±3.4 (9)	8–17
dc-dc × dc-dc	7.9±2.6 (38)	5–19
cd-cd × cd-cd	9.4±3.7 (13)	6–17
cc-cc × cc-cc	5.0±0.66 (10)	4– 6
Backcross		
P_1		
d × d	7.6±1.7 (19)	6–12
d × c	9.3±2.7 (6)	6–13
c × d	7.0±3.0 (9)	4–13
c × c	5.3±1.3 (7)	4–8
F_1		
dd × dd	12.3±4.4 (37)	7–18
dc × dd	9.2±1.8 (18)	5–12
dd × dc	13.7±6.3 (13)	6–25
cd × dd	8.2±1.7 (17)	6–12
dd × cd	13.9±4.5 (12)	8–21
cc × cc	5.7±1.6 (10)	4–9
F_2		
dd-dd × dd-dd	16.6±4.2 (6)	10–20
dc-dd × dd-dd	11.0±2.1 (16)	8–15
dd-dd × dc-dd	12.3±3.1 (10)	8–16
cd-dd × dd-dd	14.4±2.8 (17)	11–21
dd-dd × cd-dd	12.5±2.6 (14)	9–17
dd-dc × dd-dd	10.2±2.2 (12)	7–13
dd-dd × dc-dc	14.4±2.9 (11)	9–19
dd-cd × dd-dd	13.2±1.6 (17)	10–17
dd-dd × dd-cd	15.8±3.1 (9)	12–20
cc-cc × cc-cc	5.8±1.8 (9)	4–8

All experimental animals were reared under diapause-averting conditions (LD 10:14 until cocoon spinning, then LD 16:8; 24±1 °C).

*d, *C. downesi*; c, *C. carnea*; ♀ parent always indicated first.

Table 2 Colour of F_2 adult progeny from crosses between *C. carnea* and *C. downesi*, compared with the expected values in a situation involving the inheritance of a semidominant allele

	No. of adults 'True *carnea*'	'True *downesi*'	Intermediate
Observed	78	65	146
Expected	72	72	145

$\chi^2 = 1.25$; $P = 0.5$–0.8.

We propose that *C. downesi* diverged sympatrically from an ancestral population of *C. carnea*, and that it did so through the establishment of a stable polymorphism for habitat utilisation and the subsequent development of reproductive barriers between the morphs in the two habitats.

The evolution of a stable polymorphism is based on the ancestral population's occurrence in two types of habitats: the meadow–field–deciduous tree habitat to which the population was well adapted, and the coniferous habitat, to which it was less well adapted. We propose that in the *C. downesi* ancestor the mutation of gene *g* to gene *G* (the autosomal, semidominant allele that produces the dark green cryptic coloration of *C. downesi* adults) provided a selective advantage for individuals carrying gene *G* in the coniferous habitat. The selective force that favoured gene *G* in this habitat was probably the action of vertebrate and invertebrate predators. This proposal is supported by our observations (in preparation) that conifer-inhabiting species of Chrysopidae are either cryptically (dark green) coloured (as in *Chrysopa harrisii* Banks and *C. downesi*) or they produce a malodourous secretion (as in *Meleoma emuncta* Banks) that is offensive to vertebrate and invertebrate predators[27]. Therefore, in the coniferous habitat, the dark green colour of the *C. downesi* adult provided homozygous *GG* and heterozygous *Gg* adults with a selective advantage over the pale green (*gg*) form. Conversely, the light green, homozygous *gg* form retained its selective advantage over *G* individuals in the original habitat. The two homozygous forms, *GG* and *gg*, were therefore virtually restricted to separate habitats; whereas heterozygous *Gg* individuals, with their intermediate coloration, were at a competitive disadvantage in both habitats. Thus the population was subjected to disruptive selection. This selection presumably led to the establishment of a stable polymorphism involving light and dark green colour forms—each in its own habitat. Genetically-controlled preferential mating was not necessary to this step in the speciation process because disruptive selection maintained the balanced polymorphism even if both colour forms remained within a single random-mating population. But the frequency of pairings between *GG* and *gg* was probably low because of the restriction of adults of each colour form to their separate habitats.

The inheritance of *C. downesi*'s dark green colour is not unlike that proposed for early melanic forms of *Biston betularia* (L.). Kettlewell[28] deduced that the original *B. betularia* melanic form resulted from a single, semi-dominant allele which later became dominant. In contrast to the polymorphism in the ancestral *C. downesi*–*C. carnea* population, the balanced polymorphism in *B. betularia* is based on heterozygote superiority, a situation that would allow the evolution of dominance modifiers[29] and the maintenance of interbreeding between morphs. We propose colour change rather than alteration in habitat choice as the initial step leading to speciation of *C. downesi* because it allows the establishment of a stable, habitat polymorphism based on a single gene system. In contrast, alteration of habitat choice would have been more complex because at least two gene differences would be needed to establish a stable polymorphism.

Once a stable polymorphism was established, two selective forces probably favoured the evolution of the univoltinism of *C. downesi*, the characteristic that serves as the primary reproductive barrier between the forms. First, univoltinism increased the adaptation of the coniferous form to its habitat, and second, it served to reduce or eliminate the wasteful production of the less fit heterozygotes. In contrast, the multivoltinism of the original form continued to be favoured in the original habitat. During summer, the number of conifer-inhabiting predators, such as other green lacewings (*C. harrisii*[30] and *M. emuncta*) and other arthropods (hemerobiids, syrphids, hemipterans, spiders, mites), increases, whereas available prey decreases (C.A.T. and M.J.T., in preparation). Therefore competition among conifer-inhabiting predators seems to be very intense during summer and we conclude that in the *C. downesi* ancestral form selection favoured a univoltine seasonal cycle that included reproduction in early spring, larval occurrence in mid to late spring, and reproductive diapause during summer, autumn and winter. This reproductive cycle would not have been advantageous to the original, non-coniferous form because in its habitat, prey were available throughout spring and summer.

Single allele differences at two loci underlie the seasonal asynchrony of *C. carnea* and *C. downesi*[17]. Thus, the mutation of the recessive '*downesi*' alleles at these two loci and their replacement of the dominant '*carnea*' alleles in the dark green coniferous form, resulted in the evolution of the seasonal reproductive barrier between *C. carnea* and *C. downesi*. We represent the mutation of the two diapause-controlling alleles from '*carnea*' to '*downesi*' as $D_1 \rightarrow d_1$ and $D_2 \rightarrow d_2$.

The evolution of the unicyclic *C. downesi* seasonal cycle probably took place in two separate phases: (1) the addition of the aestival diapause and (2) the extension of the autumnal diapause through winter. The summer diapause conceivably reduced the incidence of interform pairings by approximately two-thirds, because two of *C. carnea's* three annual generations reproduce during summer. The extension of the autumnal diapause through winter probably eliminated the interbreeding of overwintering populations by delaying sexual maturation in *C. downesi* in relation to *C. carnea*. We propose that each of the two diapause-controlling loci regulates one of these two differences in the seasonal cycles. Recessive '*downesi*' alleles (d_1d_1) at one locus probably result in diapause induction in long-day conditions, and this locus functions in the expression of the aestival diapause. Recessive '*downesi*' alleles (d_2d_2) at the second locus probably act in maintaining diapause until after short days are experienced and they therefore serve to prolong diapause through winter. We have no evidence showing which of the alleles (d_1 or d_2) first mutated and/or replaced its dominant counterpart in the coniferous form. The '*downesi*' alleles were strongly selected against in the original non-coniferous form because of their negative effect on the reproduction rate Even one d_1 or d_2 allele at one of the diapause-controlling loci lengthens the preoviposition period (Fig. 1), which, in the original habitat would be clearly deleterious. Thus, strong disruptive selection resulted in the replacement of the dominant '*carnea*' alleles by the recessive '*downesi*' alleles in the coniferous form. As a result. the seasonal cycles of the two forms became asynchronous, interbreeding between the forms ceased, and *C. downesi* was established as a species.

Subsequent to or concurrent with the evolution of reproductive isolation, at least two types of changes increased *C. downesi's* fitness in the coniferous habitat: (1) the evolution of habitat selection and (2) modification of the thermal requirements for development. Those *C. downesi* individuals that recognise and remain in the coniferous habitat would have a higher chance of leaving offspring than those that choose the non-coniferous habitat or that choose their habitat at random. This behaviour is probably genetically controlled, but the numbers and types of genes involved are unknown.

The second change in *C. downesi* was the alteration in the lower thermal threshold (t) for growth and development. *C. downesi* from Ithaca has a slightly (~2 °C) but significantly higher t value than *C. carnea* from the same area[18]. In nature, this slightly higher threshold acts as a precise fine-tuner of *C. downesi* univoltinism.

Genetic model

Using our data from *C. downesi*, we present a genetic model for sympatric speciation through habitat diversification and seasonal isolation by non host-specific animals. Our model begins with a hypothetical ancestral species that is well-adapted to several habitats. This species is neither restricted to a particular host, nor confined to a particular plant association. Occasionally it occurs in a secondary habitat to which it is less well adapted. Also, it is multivoltine and reproduction encompasses a broad period of the annual cycle.

Step 1, the establishment of a stable polymorphism in a two-habitat situation, is similar to the first step in Maynard Smith's general model[7] for sympatric speciation. It begins with the mutation of gene a to gene A, a semi-dominant allele that confers a selective advantage on homozygous AA individuals in the secondary habitat. Disruptive selection acts in favour of aa individuals in the original habitat, in favour of AA individuals in the secondary habitat, and against Aa individuals in both habitats. Thus, a balanced polymorphism involving the A and a alleles that adapt individuals to differing habitats is established even if both morphs remain in a single, random-mating population. Assortative mating, if it does occur in the two forms because of their habitat differences, would probably not have a genetic basis.

Step 2, the evolution of seasonal isolation, involves the non-simultaneous mutation and selection of diapause-controlling alleles that restrict the seasonal period of reproduction in the new form. In our model, recessive alleles (b and c) at two unlinked loci act individually or in combination to produce a restricted (or unicyclic) seasonal cycle. Selection pressure for the b and c alleles in the new morph comes from two sources: (1) by synchronising reproduction with favourable seasonal conditions in the new habitat, the alleles increase the adaptation of the new AA form, and (2) by reducing the incidence of interform pairings they reduce the production of the less fit Aa heterozygotes. Because the original, multivoltine seasonal cycle is well adapted to the original habitat, selection acts to keep the recessive b and c alleles out of the aa population; this selection is enhanced if the diapause-controlling alleles in the heterozygous condition also produce unfavourable effects. Disruptive selection such as this leads to the establishment of two seasonally-isolated populations—the original $aaBBCC$ in the original habitat and the new $AAbbcc$ in the new habitat. With the completion of this process, the new species is established.

Step 3 in our model comprises changes that increase the fitness of the newly-formed species. For example, selection favours individuals of the new species that recognise and choose the 'correct' habitat. Therefore, genetic changes associated with the physiology and behaviour of habitat selection are likely to occur. Selection also favours changes that fine-tune the seasonal isolation of the two species and that increase the new species' synchrony with favourable seasonal conditions in its habitat. The physiological characteristics involved in these changes are usually under polygenic control[26,31,32].

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letters to nature

Variable mid-latitude X-ray source 3U0042+32

THE faint high latitude X-ray sources are a mixed population of galactic and extragalactic objects. Identified extragalactic X-ray sources include clusters of galaxies, Seyfert and other active galaxies, quasars, and nearby normal galaxies[1,2]. Identified galactic sources with $|b^{II}| > 20^\circ$ include several X-ray binaries, two in globular clusters —M15 (ref. 1) and NGC1851 (ref. 3)—the hot white dwarf HZ43 (ref. 4), the dwarf binary AM Her (ref. 5) and the flare star UV Ceti (ref. 6). The high-latitude sources generally lie in fields of low obscuration and density of objects so that the prospects for their identification with optical or other counterparts are relatively good, given position measurements of moderate accuracy. We report here a precise celestial location (1′ error radius) for one such X-ray source, 3U0042+32 (ref. 1), that is situated at mid-galactic latitude ($b^{II} \sim -30^\circ$). A study of the error box for this source indicates that it may be a distant galactic binary system similar to Her X-1.

In early February 1977, Ricketts and Cooke[7] reported a flareup in the intensity of a source in the region of 3U0042+32. Their observations, carried out with the Ariel V X-ray satellite, showed that this source, which had been below the level of detectability during a total of 50 d of scanning since November 1974, was variable up to 25 Uhuru counts per s during 3–6 February, 1977 and up to 50 Uhuru counts per s during 14–17 February. Variability in 3U0042+32 had previously been reported during the interval 1971–73 (refs 8 and 9).

Following receipt of a private communication of the Ariel-5 observation, the SAS-3 X-ray observatory was oriented to bring the flaring source within the field of its rotation modulation collimators. At the time, the location of the Sun was such as to restrict the period of useful star tracker data to only 20 min per orbit. Nevertheless, sufficient exposure was obtained during 25–26 February, 1977 to enable us to make position measurements with the data from both the 2.3 and 4.5 arc minute collimators and in two separate energy channels.

The characteristics and use of the rotation modulation collimator detectors on SAS-3 have been described previously by Doxsey *et al.*[10] and Schnopper *et al.*[11].

Table 1 Rotating modulation collimator positions* for 3U0042+32

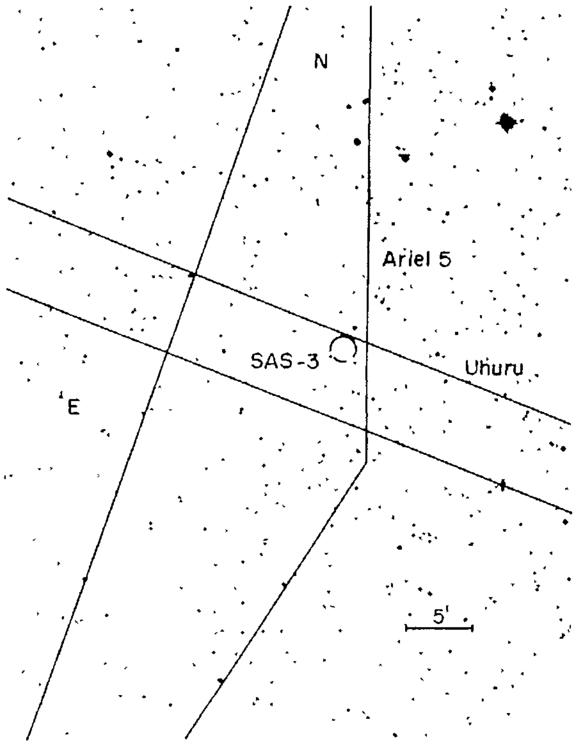
	2.3′ FWHM collimator	4.5′ FWHM collimator
Data set 1	α = 00h42m10s δ = 32° 45′ 06″	α = 00h42m06s δ = 32° 44′ 45″
Data set 2	α = 00h42m09s δ = 32° 44′ 57″	α = 00h42m09s δ = 32° 44′ 43″
Average	α = 00h 42m 08.5s δ = 32° 44′ 53″	

*Epoch 1950.0

Table 1 summarises the results on the position derived from the two independent modulation collimators. For each collimator the data were subdivided into two parts, representing the first and second halves of the observation. The mean position obtained from the combined results is α = 00 h 42 m 08.5 s; δ = 32° 44′ 53″ (epoch 1950.0) with an error circle of 1′ radius (90% confidence).

Figure 1 is an enlargement of the red Palomar Sky Survey print of this region with the SAS-3 error circle superposed on it. Also shown are the error box of 3U0042+32, the intensity of which is listed as 7.0±0.5 counts per s in the Uhuru catalogue[1], and the error box of the flaring source studied with Ariel V. It is likely that all three observations pertain to the same object, and that it lies inside the SAS-3 error circle. Within or very near this circle, there are seven stellar images above the plate limit and with visual magnitude ≲ 15.5. Harris *et al.*[12] have studied this region in detail at both radio and optical wavelengths. They point out only one weak radio source in the

Fig. 1 Enlargement of the red Palomar Sky Survey print of the region near 3U0042+32 (copyright National Geographic Society). The solid lines represent portions of the Uhuru (3U) and Ariel V error boxes[1,7] for this source. The 1 min radius error circle is derived from data obtained with the rotation modulation collimators on SAS-3 (see text).



vicinity of the SAS-3 error box (~ 2.2′ from the centre). Bahcall *et al.*[13] have also searched for the optical counterpart to this source, but without success.

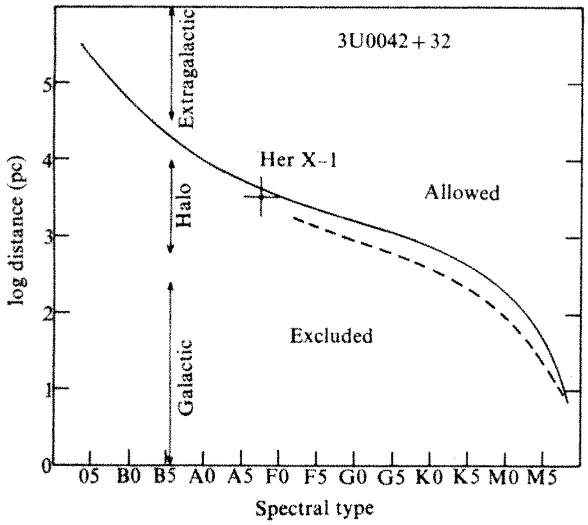
At the conclusion of the position measurement SAS-3 was switched to the fast timing mode (8 ms resolution; see ref. 14). The source was studied for regular pulsations in the range 16 ms to 20 s. Analysis of data from three satellite orbits yielded an upper limit to pulsing in this range of ~ 15% of the steady source intensity (~ 35 Uhuru counts per s). During 27.3–27.7 February, 1977, the *y*-axis detectors[15] were pointed at the source in order to search for possible longer period pulsations. These results also proved negative with a pulsing limit of ~ 7% set in the range of 1 s to ~ 500 s.

Spectral data in the energy range 1–19 keV were also obtained with the *y*-axis detectors. The energy spectrum is nearly flat out to ~ 10 keV ($\alpha = 0.0 \pm 0.4$), with a characteristic cutoff energy of $\leqslant$ 1.5 keV. At higher energies the spectrum falls off rapidly with increasing energy.

The peak X-ray flux from this source (50 Uhuru counts per s) is ~ 50 times greater than the optical flux from the brightest object ($m_v \approx 15.5$) in the error circle at the time of the Palomar exposure. The timescale of its variations, according to the Ariel V observations, is of the order of days. Its spectrum resembles that of hard X-ray binaries which derive their power from accretion on to a compact star. We believe that the most likely explanation of this flaring source is that it is a neutron star in a close binary system with a companion that undergoes occasional episodes of mass transfer due to eruption or orbital eccentricity (compare ref. 16).

The limit on the optical magnitude of the stellar companion ($m_v \gtrsim 15.5$) places significant constraints on the distance to 3U0042+32 for various assumed spectral types. These distance constraints are summarised in Fig. 2. For each spectral type the absolute visual magnitudes of a main sequence dwarf and subdwarf star[17] were used; any larger stars would require still larger distances. A companion star earlier than spectral type B5 would probably lie beyond the galactic halo. Stars of very early type are likely to be excluded, in any case, because a comparison of the Palomar red and blue plates indicates that none of the stellar images within the SAS-3 error circle exhibits a large blue excess. Only stars of type ~ K5 or later would be consistent with a location within ~ 200 pc of the galactic plane. A distant late A or early F star, similar to HZ Her[18,19] (= Her X-1) would be consistent with all presently available data on 3U0042+32.

Fig. 2 Minimuma llowed distance to 3U0042+32 against assumed spectral type of an hypothetical binary companion star. The solid and dashed curves are derived from the magnitude of the brightest star in the SAS-3 error circle ($m_v \sim 15.5$) and the absolute visual magnitudes of main sequence dwarf and subdwarf stars[17], respectively. Interstellar extinction was assumed to be negligible. Parameter values for Her X-1 (refs 18 and 19) are shown for reference.



The possibility that the source is extragalactic is remote. The peak X-ray intensity of the source is equal to that of the Perseus Cluster[1] yet there is no active galaxy or known quasar in the error circle[12,13]. The short timescale of the X-ray variations implies that the source must be 'compact' (that is, $\lesssim 10^{16}$ cm) as opposed to, for example, an extensive region of hot intracluster gas. One might then expect the spectrum of such an extragalactic object to be highly absorbed, by analogy with other compact extragalactic sources (for example, Cen A, 3C273, and NG4151; ref. 20). On this latter point, our limited spectral data may not be decisively negative. Nevertheless, if the source is extragalactic, it must be of a kind not previously observed.

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Supernovae and molecular clouds

In a study of the CO distribution in the Galaxy, Bash and Peters[1] concluded that there is an upper limit of 30 Myr to the age of stellar systems which contain CO. Younger systems, launched by the impulse of the density wave show abundant CO emission. Systems which have existed for longer than 30 Myr display no CO emission. Based on their initial observations, Bash and Peters predicted that there would be a correlation between the presence of CO emission and the turnoff in open clusters. Observations by Green confirm this prediction[2] showing CO emission in at least 24 of 28 clusters with turnoff earlier than spectral type BO and no evidence of CO emission in 35 of 38 clusters with turnoff later than BO. We suggest that there is a critical mass M_{crit} such that for $M > M_{\mathrm{crit}}$ stars do not evolve to an explosive phase and hence the surrounding molecular cloud is substantially unaffected by the evolving stars. As the system ages, the most massive star with $M < M_{\mathrm{crit}}$ finally evolves to an explosive endpoint and the resulting supernova dissipates the dense molecular cloud. From the cluster observations we identify $M_{\mathrm{crit}} \sim 15\text{–}20 M_{\odot}$, the mass of a BO star (ref. 3 and E. Green, private communication), although there is some uncertainty in luminosity class.

An alternative is that one is merely seeing an excitation phenomenon, that stars with $M > M_{\text{crit}}$ are necessary to excite the CO molecules and those with $M < M_{\text{crit}}$ are unable to excite. The basic argument against this possibility is that the disappearance of CO emission seems to be correlated with the disappearance of HII regions as well[2]. HII and CO regions have very different excitation characteristics and, hence it seems unlikely that they could both be made to vanish simultaneously by altering a single source of excitation.

A crucial point to the present argument is that a supernova be able to disrupt the molecular cloud. Molecular clouds have mass up to $\sim 10^5 M_\odot$ and density $\sim 10^4$ cm^{-3}. Such large clouds have a gravitational binding energy of $\sim 2\times10^{50}$ erg and an atomic/molecular binding energy $\sim 3\times10^{51}$ erg. Thus, a healthy supernova should be able to disrupt a cloud as massive as this if all its energy is utilised for this purpose. Mouschovias[4] argues, however, that a supernova in such a cloud will pass from the adiabatic Sedov phase to the isothermal, or snowplow, phase long before the shock produced by the supernova reaches the surface of the cloud. If this were the case only the momentum at the onset of the isothermal phase, not the energy of the supernova, would be available. The initial momentum of a typical supernova, $(2M_{\text{SN}}E_{\text{SN}})^{1/2}$, is capable of giving an outward velocity of $v \sim 3.2$ km s^{-1} $((M_{\text{SN}}/10M_\odot)E_{51})^{1/2}M_{\text{cl},4}{}^{-1}$. The escape velocity is $v_{\text{es}} \sim 4.5$ km s^{-1} $(M_{\text{cl},4}{}^{1/3}\, n_4{}^{1/6})$. Thus, a $10^4\ M_\odot$ cloud can be disrupted by a supernova of $\sim 20\ M_\odot$, roughly the mass we are considering.

Molecular clouds, like stars, follow a fairly steeply decreasing mass function. While the details are not fully agreed on, one can argue that a typical cloud probably contains of order $10^4 M_\odot$; that the $10^5 M_\odot$ clouds are observationally striking, but not representative. In particular, $10^4 M_\odot$ is probably a more typical mass for the molecular clouds surrounding the young open clusters with which we are especially concerned. Thus we conclude that supernovae are the likely cause of disruption of most molecular clouds, even if only their momentum, not their full energy is utilised.

For a $10^4 M_\odot$ cloud with uniform density of 10^4 cm^{-3} the radius (R) is 2.2 pc, the dissociation energy is $\sim 3\times10^{50}$ erg and the binding energy $\sim 4\times10^{48}$ erg. A supernova of $10M_\odot$ and 10^{51} erg will sweep up its own mass entering the Sedov phase at $R \sim 0.2$ pc and $t \sim 30$ yr. This adiabatic phase will end at ~ 0.5 pc and ~ 300 yr when the radiation cooling time drops below the expansion timescale. The rapid leakage of radiation will begin at temperature, $T \sim 1\times10^7$ K for these high densities and be further enhanced at $T \sim 10^6$ K when recombination radiation from heavy ions becomes very strong. An HII region will then race ahead of the original shock front dissociating the cloud. This will lead to a loss of thermal energy and eventually the ionisation energy as the cloud expands and recombines. For a $10^5 M_\odot$ cloud the HII region is probably unable to totally dissociate the cloud. On recombination, the Lyman α flux will be re-radiated from grains as a burst of 10 μm radiation which will escape from the cloud.

Thus while the details are different because of the dense cloud, the evolution of the explosion may be qualitatively similar to the standard picture[5] and we conclude that Mouschovias' arguments concerning the energetics are basically correct. A more careful treatment would incorporate possible density gradients in the cloud. A decreasing density outward, as is observed in many clouds, might serve to prolong the Sedov phase. A step function from low to high density due to a pre-existing H II region would lead to reflected shocks and perhaps an elimination of the already rather brief Sedov phase. These details are presently under consideration in a program which will lead to detailed numerical calculations.

In the simplest picture, the longest lived very bright phase, and hence the one most likely to be observed, may be the Sedov phase. There is an appreciable flux of radiation in this phase, primarily thermal bremsstrahlung, even though it is not sufficient to effect the shock dynamics. This flux of soft X rays will probably be absorbed on grains, raising them to several hundred K and again will be seen in the 10 μm range. The estimated flux is $\sim 5\times10^6 L_\odot$ for a time of about 100 yr. If supernovae occur in our Galaxy with a mean time interval $\lesssim 40$ yr then the present number of events could be $\gtrsim 3$ if they each last ~ 100 yr. Kwan and Scoville[6] have suggested that the Becklin–Neugebauer object may be the site of an explosion.

We have suggested in this note that there exists an upper limit to the mass of stars which explode, ~ 15–$20M_\odot$. This upper limit might exist because of differences in internal structure at the state just before iron core collapse in the final stages of evolution. This possibility leads to the suggestion that stars earlier than BO do not explode because they collapse to make black holes, a notion which is consistent with a suggestion made previously by Wheeler and Shields[7]. Alternatively, these massive stars are known to lose mass and the possibility exists that their mass is reduced to that of a BO before significant evolution from the main sequence can occur (T. Mazurek, personal communication). In this case, the changes in the internal structure, if any, are unclear.

In either case, if this conjecture about an upper mass limit to supernovae proves to be true, quantitative, but perhaps not qualitative, changes might have to be made in notions of nucleosynthesis. Stars from, say, $10M_\odot$ to $20M_\odot$ would still be available as sites of explosive nucleosynthesis and, because of the steep mass function, these are most of the stars which would contribute anyway.

Another important ramification of this suggestion is that star formation would cease with the supernova explosion and the dissolution of the cloud. This picture thus accounts very naturally for the observed inefficiency of star formation in molecular clouds.

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Molecular images by electron-wave holography

IMAGES of electron clouds in gas-phase atoms have been photographed[1,2] at a resolution limit of 0.1 Å through a two-stage holographic microscope based on Gabor's principle of image reconstruction[3]. Information encoded on to holograms with electron radiation in the first stage was transformed into a portrayal of electron density in the second stage with the aid of an optical laser. Suitable pictures of gas-phase molecules could not be obtained because of signal-to-noise problems. These problems have now been overcome to a considerable extent and we report here the reconstruction of spherically averaged molecular images displaying light atoms in the vicinity of heavy atoms. Bond lengths can easily be measured with a ruler.

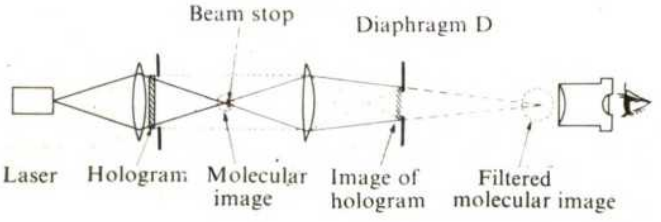
To make holograms yielding high resolving power, it is necessary to produce a broad reference wave that can be mixed coherently with waves emanating from the object under scrutiny. Stringent mechanical stability is also essential. Optical path length differences between the reference beam (source to detector) and object waves (source to object to detector) must not vary during recording by more than a fraction of a wavelength. To achieve these requirements in optical holography, great care must be taken. To achieve them with electron holography, where wavelengths are only one 1/100,000 as long, would be virtually impossible unless the reference beam diffuser were locked internally in the molecular system to be viewed. In atomic holography this was accomplished very naturally for electron cloud objects by scattering the incident wave from the atomic nuclei associated with the electron clouds. In this study of molecules a heavy atom in each molecule served to scatter a strong reference beam. In both cases, then, each particle in the sample to be investigated carries around its own reference wave diffuser, as it were, and produces its own hologram. Holograms from all particles in the sample fall on top of each other. Therefore, the reconstructed images also all fall on top of each other. Instead of seeing individual atoms or molecules we see the average of an ensemble. If all members are identical the images are not degraded. Because our molecular objects are in the vapour phase to reduce extraneous scattering, they have chaotic orientations. Accordingly, we see only the spherical average. This might seem disappointing, but it should be noted that any individual, free molecule in any given quantum state is inescapably diffuse in orientation, and spherically delocalised in the ground quantum state.

For our first molecular investigation we chose AsF_5, an example where five light 'object' atoms (fluorine) are packed with D_{3h} symmetry around a central heavy atom (arsenic). What we should expect to see and do in fact see in the reconstructed image is a spherical shell of atomic fluorines. The radius of this shell corresponds to the mean As–F bond length.

For the first stage of our holographic microscope we used the apparatus previously used for atoms[2], but we reduced the numerical aperture twofold, to $f/2.3$. Resolving power was sacrificed to record with maximum faithfulness the strongest holographic interference fringes. Holograms were recorded on Process plates by scattering 40-kV electrons ($\lambda = 0.06$ Å) from a fine jet of AsF_5 vapour at 20 torr and filtering the electrons through an r^3 rotating sector. This filter is deliberately weaker than the 'Rutherford filter'[2] adopted for monatomic samples to bring out the detail of the light atoms more strongly. Once obtained, the holograms were reduced 18-fold by rephotographing on Kodak 120-02 holographic plates.

Rendering the light atoms visible in the presence of the heavy central atom required a new design for the second, reconstruction stage of the microscope. As before, the desired image is a component of the Fraunhofer diffraction pattern of the hologram. But the holographic fringes corresponding to the As–F bonds are comparatively subtle, and the reconstructed image of fluorine atoms lies very close to the intense 'undiffracted' zero-order beam transmitted by the hologram. (The radius of the molecular image occurs at about the tenth ring of the Airy diffraction pattern produced by the holographic aperture. The beam stop illustrated in Fig. 1 screens through the third Airy ring.) If left at its natural level, this zero-order beam overwhelms the desired image. In principle, the most efficient way of getting rid of the unwanted beam without distorting the molecular image would be to cancel it with another, properly modulated, beam in a Mach–Zehnder interferometer[2]. In practice, it proved much simpler and adequately effective to use a technique suggested by Professor Emmett Leith, namely to remove the zero-order beam differentially by the 'spatial domain filter'[4] illustrated in Fig. 1. An internal calibration of the magnification afforded by the second stage of the microscope could be made easily by measuring the interference spots produced by a 100 line per inch Ronchi grating placed over the hologram.

Fig. 1 Schematic drawing of optical reconstruction stage of holographic microscope. The unwanted beam passing undiffracted through the holographic fringes is largely screened out by the beam stop; most of the remainder is skimmed off by diaphragm D which passes the image-forming rays.



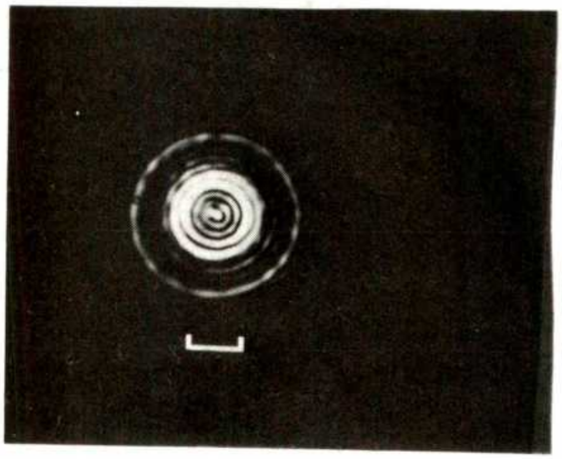


Fig. 2 Holographic image of rotational average of AsF_5 molecules at 70 million power; spherical shell of fluorine atom density plainly visible at 1.6 Å radius. Scale bar 1 Å.

A reproduction of an image of the rotationally averaged AsF_5 molecule at an Abbe resolution limit of 0.2 Å is shown in Fig. 2. Because of the curvature of the spherical shell of fluorines, the maximum density of the projected image lies a few hundredths of an angstrom unit inside the actual mean bond length of 1.68 Å (ref. 5). In principle it would be possible to deconvolute the projected density in Fig. 2 and determine the amplitude of vibration of the As–F bonds from the breadth of the fluorine shell, but this has not yet been done. Since the computed[2] depth of focus of our microscope at the numerical aperture used is about 4 Å and, hence, is appreciably greater than the As–F distance, the three-dimensional aspect of the spherical image is largely lost. As is the case in conventional holography there are 'extraneous contributions' to the image arising from incoherent scattering and from the square of the object amplitude. In heavy exposures the latter contributions show up as shells revealing the $F\cdots F$ non-bonded distance occurring at $\sqrt{2}\, r_{AsF}$, and vestiges of the non-bonded distance at about $\sqrt{3}\, r_{AsF}$. Indeed, given a molecule without a single dominant heavy atom, the laser reconstruction would yield series of concentric shells the profile of which would be closely related to the radial distribution function of internuclear distances in the molecule.

For many years electron diffraction patterns have been measured with microdensitometers sensitive to changes in absorbance of a few parts per ten thousand. Molecular structures have then been deduced by computer analysis of diffracted intensities. In our technique, a much more direct display of structural information is provided immediately by the optical Fourier spectrum of the electron hologram. Although the new technique is not yet competitive in accuracy with the well established scattering techniques, it may find a useful role. Whether analogous techniques can be worked out to study oriented molecules is a challenging problem for the future.

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Formation of silver mirror by photolysis of thin films of high molecular weight silver compounds

SILVER salts of carboxylic acids such as formic acid, acetic acid and oxalic acid[1] became coloured in light. Similarly, suspensions of silver polyalkylacrylates produce various coloured colloidal solutions of silver in light[2]. These phenomena show that silver carboxylates are easily photolysed to separate silver. We have investigated the photolyses of silver salts of certain high molecular carboxylic acids such as polyacrylic acid, polymethacrylic acid, poly-L-glutamic acid[3], alginic acid[4], pectic acid[5], carboxymethylcellulose and cellulose acetate phthalate, and we report that the smooth films of these silver salts form a silver mirror arising from photolytic silver on their surfaces. This method of producing a thin silver film on the polymer film may be of technical interest because of the optical and electrical properties of the irradiated film.

Thin films of high molecular weight silver carboxylates (about 4 μm thick) were prepared in the following way. The aqueous solutions of the sodium salts of about 0.5—1% (1 ml) were coated on 20-cm^2 glass plates and dried in the air, then the plates were immersed in silver nitrate solution of 0.1 M for 1 h and washed with water to eliminate sodium nitrate and excess silver nitrate contained in the films, and dried again in the air. For preparation of films of silver salts with smooth surfaces it was essential that the aqueous solutions of sodium salts were dried sufficiently to form the films of sodium salts on the glass plates. This technique thoroughly converted the films of soluble sodium salts into films of slightly soluble silver salts by a rapid ion exchange reaction between sodium ions and silver ions in the films. Thus, the amorphous films of silver salts are prepared on the glass plate, and, if necessary, can be easily peeled from the plate.

The films of all silver salts are colourless and transparent, and show a broad absorption band between 200 nm and 300 nm, which is associated with the charge transfer from carboxylate ions to silver ions. On irradiating with ultraviolet rays (200–300 nm), the films, at first, became brown coloured ($\lambda_{max} = 425$—460 nm) due to the formation of photolytic colloidal silver particles in the films. When irradiation was continued, however, the irradiated surfaces of the films gradually assumed metallic lustre and finally changed into clean silver mirrors. Because of the formation of silver mirrors, the surfaces of the sufficiently irradiated films had very high electric conductivities, which were about 10^9 times as high as those of the original films.

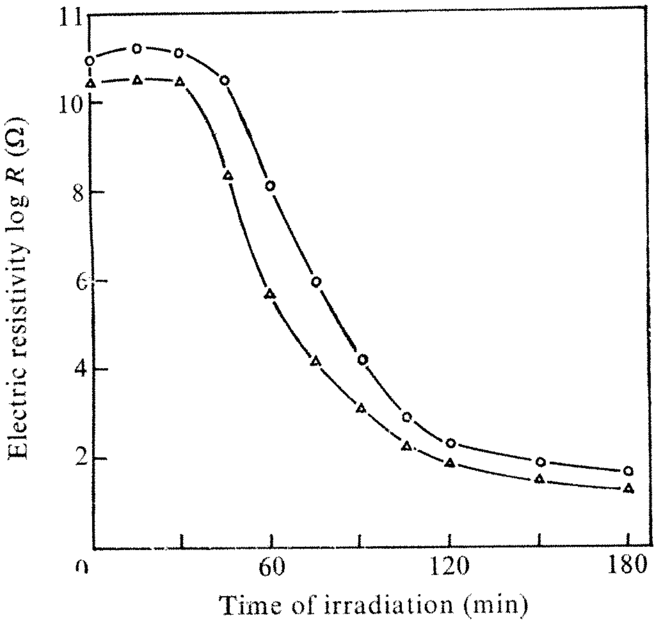

Fig. 1 Plot of log resistivity of the irradiated surface against time of irradiation for silver polyacrylate (○) and silver carboxymethylcellulose (△). The films, about 8μm thick, were exposed to ultraviolet rays from a sterilisation lamp at a distance of 3 cm in air of relative humidity 80% at 20 °C. The electric resistivity (measured in ohm) is that of the irradiated film surface of 1 cm^2. In the electrical resistance measurements, two silver plates (1 × 1 cm) were pressed on to the irradiated surface, at 1 cm apart, with a constant force. Since the photolytic silver existed predominantly on the irradiated surface and a little in the interior of the polymer film, the resistances between the irradiated surface and the rear surface, and of the rear surface scarcely changed with irradiation.

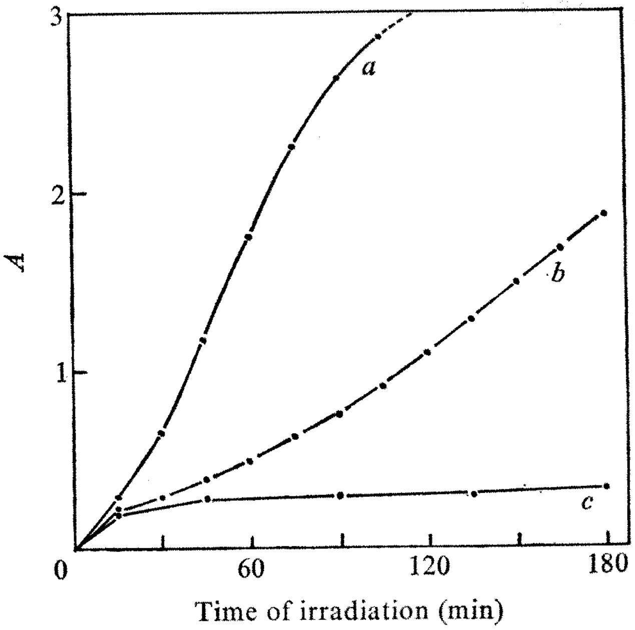

Fig. 2 Effect of water on the separation of silver for carboxymethylcellulose. The films were exposed in various humidities at 20 °C. The absorbance (*A*) was measured with a photographic densitometer. Chemical analysis confirmed that the optical density was proportional to the amount of the photolytic silver in the film. Humidities were, line *a*, 80%; *b*, 59%; *c*, 0%.

Figure 1 shows a plot of log resistivity of the irradiated surface of 1 cm^2 against time of irradiation for silver polyacrylate and silver carboxymethylcellulose. Similar results were obtained for the other silver salts in suitable conditions of irradiation. Since it is clear that the photolytic product which gives high electric conductivity on the surface is metallic silver, it is suggested that silver atoms produced by irradiation aggregate to form the colloidal silver particles near the irradiated surface and finally form a thin layer of metallic silver on the surface[6,7].

Water has a very important role in these photolyses. When irradiation was carried out in a dry box, the separation of silver occurred very poorly even on the prolonged irradiation (Fig. 2), and hence the surface of the film did not exhibit the metallic lustre. To form a silver mirror and increase the electric conductivity on the surface, it is necessary to irradiate the film under an atmosphere of the high relative humidity. It is clear that the water content in the film is closely related to the relative humidity in which it is placed. It seems likely, therefore, that the effect of water results from the action of water as a plasticiser, which facilitates the segmental motion of the polymer and restrains the recombination of the primary products, that is, silver atoms and acyloxyl radicals.

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Particulate transport through the mesosphere and stratosphere

THE 'aerosol layers' frequently observed at about 50 km altitude may be due to downward transport from above of particulates, possibly of extraterrestrial origin. This transport would be more efficient at higher altitudes (the mesosphere) than lower altitudes (the stratosphere) thus causing the layering seen in the stratopause region. The flux of particulates through the stratosphere provides a potential scavenging mechanism for pollutants and a source of condensation nucleii to the troposphere. Electric field variations, probably induced from above, can modulate this flux, providing a possible coupling mechanism between geomagnetic activity and terrestrial weather. The particulate distribution is also affected by the general circulation and atmospheric dynamics. The density of particulates is much greater than generally recognised, indicating that their role in chemical and thermal processes should be reassessed.

Noctilucent clouds provide visible evidence of the occasional presence of particulate matter in the high latitude summer mesopause region, but there is also evidence for the continual presence at many latitudes of a high density of very small (<10-nm) aerosol particles in the mesosphere and stratosphere. This evidence includes unexpectedly low and variable ion mobility and conductivity observations (refs 1–6) and ionospheric data such as the normally low density of electrons in the mesopause region[4] and the very rapid loss of electrons during solar eclipse totality[7]. Features of the 'winter anomaly' in ionospheric radio wave absorption, such as the large variability[4] and solar zenith angle dependence[4] of electron density, can be explained by the absence of these particulates on 'anomalous' days.

Optical measurements, using a variety of techniques (Lidar, rocket and satellite scattering measurements at various wavelengths), have indicated 'aerosol layers', frequently in the vicinity of 50 km (ref. 8 contains references to earlier observations). This layering in the stratopause region may be explained by the more effective downwards transport in the mesosphere than the stratosphere of particulate matter, which may be of cosmic origin, possibly coated with ice or water[4]. Gravitational settling of 10-nm particles is less than 10^{-1} m s^{-1} at 80 km, decreasing rapidly at lower altitudes[9]. The dominant diffusion process in the stratosphere and mesosphere is 'eddy diffusion' and the applicable diffusion coefficient is much higher in the mesosphere than the stratosphere, which could produce layering due to 'piling up' in the transition region, the stratopause. The expected eddy diffusion coefficient for the mesosphere[10] yields characteristic downward velocities of order 10^{-2} m s^{-1}, implying a trans-mesospheric transit time of order 30 d. A more rapid process might involve the vertical electric field, which is generally expected to be downward in the upper atmosphere[11]. To provide a net downward transport there must be an excess of positively- over negatively-charged aerosol particles (or a differential mobility). An excess of positively-charged particles would be expected since free electrons form in this region due to detachment (or emission) from negative ions. We need not rely on speculation on this point, however, since Widdel *et al.*[2], using a parachute-borne Gerdien condenser, observed positive ions in the mesosphere with mobilities an order of magnitude less than that of the small positive ions, but did not observe the corresponding low mobility negative ions, indicating that negative particulates did not form, or, more likely, were of very short lifetime due to electron detachment. A consensus of the properties of the particulates in the mesosphere[4] would indicate a density of 10^9–10^{10} m^{-3} of less than 10 nm diameter and a mass of several thousand AMU. Recent measurements of the vertical electric field indicate a field of the order 1 V m^{-1} in the stratosphere, with some intensification in the mesosphere[12]. Assuming a 1-Vm^{-1} field and mobilities for the particulate ions as for the lower mobility group seen by Widdel *et al.*[2], of 1 m^2 V^{-1} s^{-1} at 70 km (10^{-1} at 60) yields velocities of the order of 1 m s^{-1} at 70 km (10^{-1} at 60). Since at least 10% of the available aerosol particles can be expected to be charged (deducible from refs 2, 4, 13), this will provide a transport mechanism at least comparable with eddy diffusion if the electric field measurements are correct.

Recent measurements by Hirono *et al.*[14], using both Lidar and direct electrical measurements on balloon-sondes, indicate a population of 'Aitken' particles in the stratosphere having similar densities ($\sim 5\times10^9$ m^{-3}) to those deduced above for the mesosphere, with a relatively constant density (to a factor of 2) from the tropopause up to 30 km. The eddy diffusion coefficient is much smaller in the stratosphere[15] and the trans-stratospheric transport by this mechanism would be expected to take years. The density of particulates would imply a downward flux of the order of 10^6 m^{-2} s^{-1}. The corresponding electric field transport mechanism is hindered in this region by much lower mobilities (inversely proportional to air density), and is dependent for net transport on a differential density or mobility effect between positively and negatively charged particulates, as before. It would be expected that there might be some differential density effect due to detachment of electrons, as in the mesosphere. Assuming a reduced (STP) mobility of 3×10^{-5} m^2 V^{-1} s^{-1} (ref. 2), the downward velocity would be 3×10^{-2} m s^{-1} at 18 km for a 1 V m^{-1} electrical field, and would be constant above this for the usual assumption that the electric field decreases exponentially with altitude in the stratosphere, consistent with the observation of a relatively uniform particulate density[14]. For a 10% net differential density of positive over negative particulates the downward flux would be of the order of 1.5×10^7 m^{-2} s^{-1} and trans-stratospheric transit would take about 10^2 d. Disturbances of the field, such as have been observed in connection with geomagnetic activity (ref. 16 and R. Mühleisen, personal communication) could modulate or interrupt the flow of condensation nucleii into the troposphere. This is the sort of mechanism which has been suggested to explain possible connections between solar and geomagnetic activity and weather[17,18].

The distribution of particulates would of course also be modified by atmospheric dynamics and the general circulation. One scenario for the 'winter anomaly' in radio wave absorption in the mesosphere would involve the depletion of particulates in sub-auroral regions by an enhanced or reversed vertical electric field, with these regions then being transported by high-velocity equatorward winds. The early winter downdraft at mid-latitudes[19] would be expected to move particulates into the troposphere. A wave-like structure in conductivity observed at a low-latitude site[5] can be explained by a wave-induced bunching of particulates.

The presence of such a high density of particulates in generally downward flow throughout the mesosphere and stratosphere shows that their role in chemical and thermal processes should be evaluated, but this remains uncertain without knowledge of their structure and composition. For example, their effectiveness in scavenging could be greatly enhanced if they are water coated.

It may be that the 'fair weather' electric field expected at the tropopause could be perturbed using high d.c. voltages on high electrodes or antennae, possibly using tethered balloons. Less than 10^6 V on an electrode 1 km above its image would produce an electric field sufficient to double or cancel the expected tropopause electric field (1–10 V m^{-1}) at any latitude. This suggests a means of testing for an interaction between the vertical electric field and atmospheric processes with Lidar or sonde measurements.

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Novel method for direct analysis of hydrocarbons in crime investigation and air pollution studies

MODERN methods for sampling of organic vapours in air pollution studies avoid the problems caused by the condensation of water vapour, by collection at ambient temperature on charcoal[1], porous polymers[2–5] or support-bonded phases[5]. The tenacity of adsorption renders the sample difficult to remove and indirect methods such as liquid extraction[1,5] or thermal desorption[2–4] are necessary to produce a sample for gas chromatographic (GC) or GC–mass spectrometric analysis. We describe here an analytical technique based on the Curie point pyrolysis system[6] which overcomes these problems by using ferromagnetic wires coated with a thin layer of highly active charcoal. These are simply suspended in the atmosphere under test for 1–2 h at room temperature, placed in the Curie point apparatus and then inductively heated in the normal manner. Thus the sample is rapidly introduced into the GC for direct analysis. The fact that the total amount of each hydrocarbon present is not adsorbed by this method is outweighed by the advantages of direct sample introduction, which offers distinct advantages to the forensic scientist in the investigation of a suspected arson. The technique could also find quantitative application in air pollution studies.

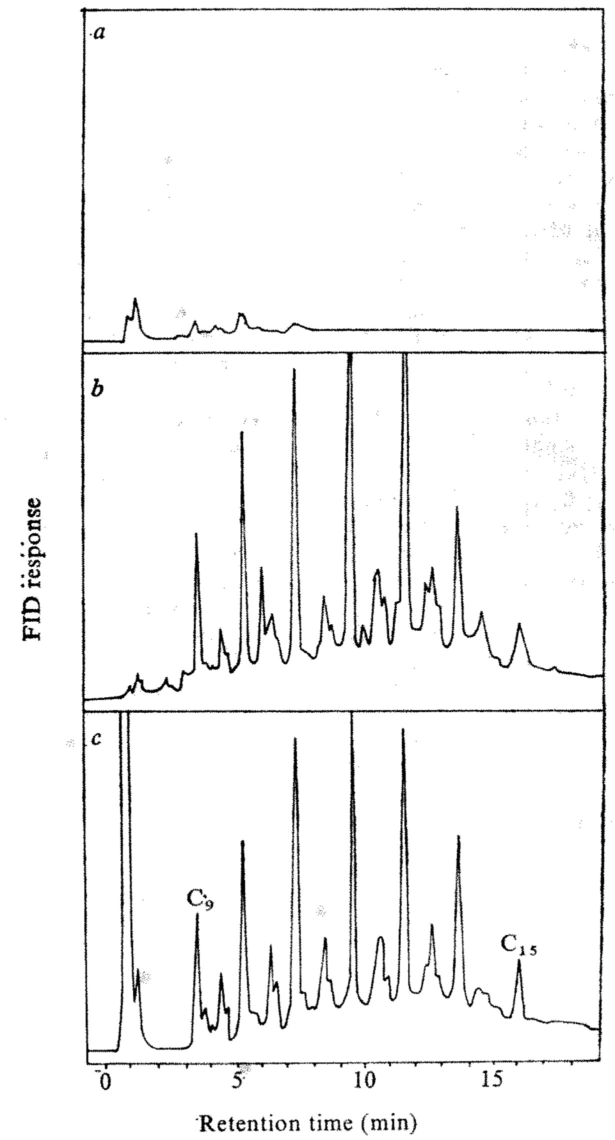

Fig. 1 Gas chromatograms obtained from *a*, 1-ml aliquot taken with heated syringe from a 180-ml glass bottle sealed with nylon film, containing 5μl paraffin and heated to 110 °C; *b*, induction heating for 10 s in the Curie point apparatus of an adsorption wire after 1.5 h immersion in an identical headspace to (*a*), at 22 °C, the chromatogram being obtained at the same attenuator settings as above; *c*, solution injection of the same paraffin sample for reference. Gas chromatography was carried out on a Pye 104 instrument fitted with flame ionisation detectors (FIDs) using 10-foot × $\frac{1}{4}$-inch columns of 15% carbowax 20M on Chromosorb W. A flow rate of 60 ml per min of nitrogen was maintained and the column programmed from 70–180 °C at 8 °C per min. Adsorption wires were prepared by bonding finely divided (passed 240 mesh) highly active carbon (Norit SX1, Hopkin and Williams) to nickel (358 °C Curie point) wires with a high temperature inert adhesive (Cement Binder LQ/S6, Fortafix Ltd). The dried wires were conditioned by several 10-s inductive heating periods in a Pye Curie point apparatus connected directly to the GC column in the normal manner. Wires are ready to use when no further peaks are observed on the GC trace with a subsequent induction heating period. After removal from the apparatus and exposure of an adsorption wire to the vapours under examination, it is returned to the apparatus. The wire is inductively heated for 10 s and the column temperature programming started. At the end of the GC run the wire can be reconditioned for reuse by further repeat inductive heating periods as above. Although 358 °C Curie point wires were chosen initially to avoid pyrolysing the adsorbed material, limited use of 610 and 770 °C Curie point wires suggests that this is not a problem with the hydrocarbon mixtures examined. It is advisable to store conditioned wires over freshly-activated charcoal.

In forensic examination of fire debris for accelerant residues, quantitation has little relevance, the main objectives being to identify the type of accelerant, and where required compare it with a control sample. Current methods rely on the relatively high vapour pressure of hydrocarbons to separate them from the debris. Headspace analysis[7], by direct injection of vapour aliquots taken from a heated nylon bag containing the debris, can often indicate the type of accelerant used. Limitations are imposed on this method by the decrease in vapour pressure of the hydrocarbon species with increasing molecular weight. Thus the less volatile accelerants give poor headspace chromatograms and depletion of the more volatile constituents during a fire creates further difficulties in the identification of the original accelerant. Other techniques involve thermal desorption of the residues followed by cold trapping and are lengthy procedures; the condensate still requiring further processing before analysis. Adsorption traps (J. B. F. Lloyd, personal communication), while improving the procedure again do not provide direct sample introduction. The carbon adsorption wire technique is eminently suited to arson investigation, as it retains the simplicity of headspace analysis whilst producing a much more representative chromatogram, especially for the less volatile material present in the sample.

Figure 1 shows comparisons obtained from identically prepared paraffin vapour samples using hot headspace analysis (Fig. 1*a*) and using an adsorption wire at room temperature (Fig. 1*b*). These comparisons are made at the same instrument attenuation in order to demonstrate the concentrative effect of the wire. The results show that to obtain a response for *n*-decane equivalent to that obtained by the wire 21 ml of hot headspace would be required. Less volatile species show further improvement; for *n*-hendecane and *n*-dodecane, 55 and 200 ml respectively of headspace would be required. Comparison of the wire chromatogram with that of the liquid sample (Fig. 1*c*) demonstrates the wire's ability to adsorb the entire range of components present in a paraffin sample producing a chromatogram closely resembling the original material. Although the adsorption wire chromatogram shows slight differences in fine structure from the liquid paraffin sample, extremely accurate reproduction occurs in the case of petrol samples at the less volatile end. This may be due to slight preferential adsorption of the aromatic species by the carbon, and presents no problems for paraffin samples providing any samples for comparison are also examined by the adsorption wire method.

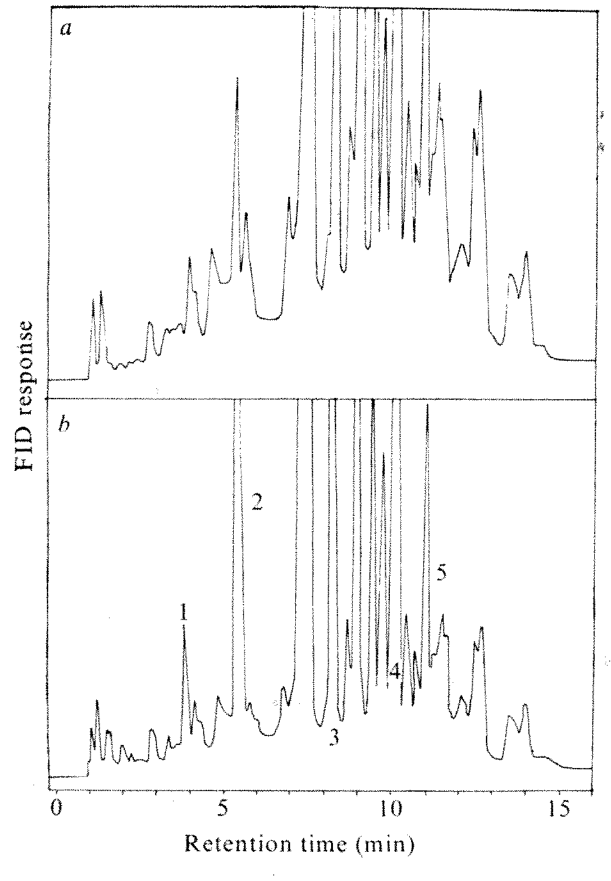

Fig. 2 Adsorption wire chromatograms obtained (as described in Fig. 1) from *a*, petrol sample from which 90% of the material had previously been allowed to evaporate in an open dish; *b*, from the original petrol sample. (Numbered peaks are referred to in Fig. 3.)

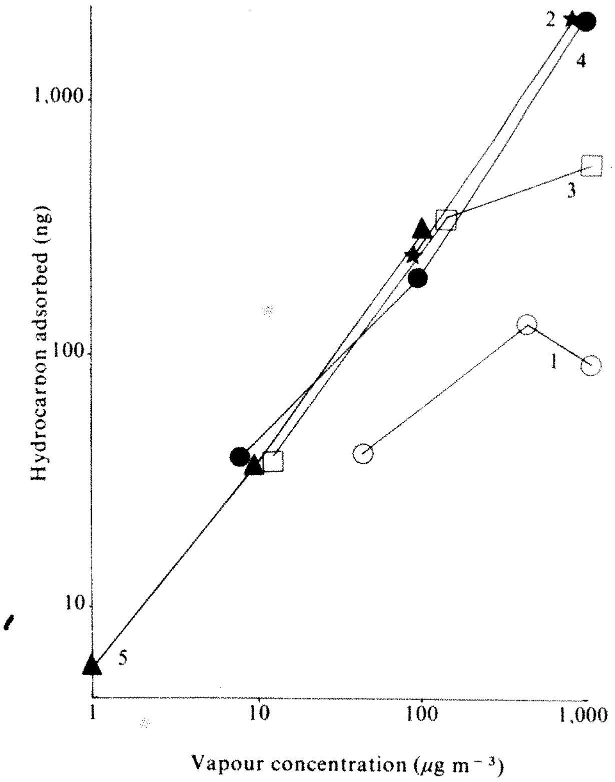

Fig. 3 Amounts collected by an adsorption wire versus vapour phase concentration for various species (log scales) after insertion for 1 h in a 100 l nylon bag containing known dilutions of petrol vapour in air. 1, Benzene; 2, toluene; 3, *o*-xylene; 4 and 5, trimethylbenzenes. Other aromatic species examined (not shown for clarity) fit the same calibration line as 2–5.

The ready adsorption of less volatile materials by the carbon overcomes the problems encountered by headspace analysis in dealing with residues which have been extensively evaporated in a fire. Figure 2 compares adsorption wire chromatograms obtained from a 90% evaporated petrol sample (Fig. 2*a*) with that from the original petrol (Fig. 2*b*). The only differences observed are at the more volatile end, the remainder of the chromatograms showing identical fine structure. This demonstrates the considerable advantages of the technique for the recognition of heavily evaporated accelerants in crime investigation.

Rapid adsorption of vapour phase components occurs with these wires, characteristic petrol traces being obtainable after only a few seconds immersion in a strong vapour. For the less volatile components of a paraffin headspace immersion for about 1.5 h is necessary to obtain a representative chromatogram, as the situation is complicated by adsorption on the vessel walls or in the debris. Where the vapours could not escape no further change has been observed in the wire chromatogram on longer periods of immersion.

The technique has been used successfully to identify petrol and paraffin residues in simulated fires with pine sawdust, when headspace analysis failed to produce recognisable traces. It has also been used as a rapid means of introducing retention index standards for calibrating Curie point pyrolysis GC. For this application the wires are held in the vapour space above each liquid sample for several seconds, separately for individual species, or sequentially for a series of materials. In this way the samples may be introduced in identical manner to that for the pyrolysis products, and changing of the inlet system is not required.

The technique has obvious application to air pollution analysis as a means of collecting and identifying components in a stack gas or other emission source. The method can be calibrated and has sufficient sensitivity for the quantitative monitoring of ambient air. Repeat experiments with the same wire immersed in equivalent volumes of known concentrations of petrol vapour in air gave the results displayed graphically in Fig. 3. Although the relationship between the amount adsorbed and the gas phase concentration was nonlinear, the adsorption characteristics of aromatic species studied were remarkably similar, with the exception of benzene. At levels exceeding $100\,\mu\text{g m}^{-3}$ (about 500 ng adsorbed on the treated wire) saturation effects began to appear for toluene and the xylenes, but below this level a calibration based on a mean line for all species (except benzene) would apparently result in errors of less than a factor of two. These results suggest that the technique may be used down to levels of $1\,\mu\text{g m}^{-3}$ which for the range of species examined is equivalent to 0.2–0.3 p.p.b. (volume parts per 10^9).

Depending on the species and site locations, city levels of these species lie in the range 0.3–130 p.p.b. (refs 1, 4, 8). Thus the method has the required sensitivity for ambient air monitoring although further work would be necessary to establish the performance of these adsorption wires in the dynamic environment of a city street.

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The Asian source of Arctic haze bands

'Arctic haze' refers to turbid layers of air which are found regularly over the pack ice north of Alaska during periods of clear weather[1]. These layers are diffuse, hundreds to thousands of kilometres wide, 1–3 km thick, and can occur as single or multiple bands of different heights at nearly any level in the troposphere. They are invisible from the ground, but may limit horizontal and slant visibility within a layer to as little as 3–8 km. Their colour is grey–blue in the antisolar direction and reddish–brown in the solar direction, suggesting that they are true aerosol rather than ice crystals.

The initial, purely visual observation of Arctic haze were made more than 20 years ago. It was then forgotten about until 1972 when radiation measurements near Barrow, Alaska revealed unexpectedly high atmospheric turbidities, confirmed in 1974 (ref. 2). The anomalous turbidity was partly found in distinct layers at altitudes of only a few kilometres. About 40%, however, was above 4 km, the effective ceiling of the aircraft used.

The 1974 observations raised the question of possible anthropogenic origin of the haze, because rough trajectory analysis for a persistent haze period during March and April 1974 suggested that the air had passed over the north-eastern United States some days earlier. To check this possibility, we have used the chemical composition and morphology of the spring 1976 Arctic aerosol as indicators of its source, and concluded that it originated in the great Asian deserts. During April and May 1976 a series of 15 flights with a single-engine aircraft were made from the Naval Arctic Research Laboratory in Barrow, Alaska. Nine high-volume (90–360 m^3) samples of haze aerosol were collected on acid-washed 11-cm Whatman No. 41 cellulose filters. Nuclepore filter samples were taken concurrently for electron microscopy. Height of the haze layers was determined by a combination of visual observation, condensation-nucleus count, and turbidity profile.

The high-volume samples were subsequently analysed at the University of Rhode Island for a number of elements with short-lived nuclides by neutron activation, with results shown in Table 1. The month-long sampling period had very low elemental concentrations at the beginning, a strong maximum in the middle, followed by a sharp dropoff at the end. The high-concentration period coincided with visible haze bands, all of whose visual properties suggested Arctic haze. The bands did not come and go quickly; rather a single broad maximum of 6–13 days' duration was seen. During this period Al and Mn were increased in concentration relative to their initial and final values by over an order of magnitude, V by an order of magnitude, and Na and Ba by somewhat less than an order of magnitude.

The aerosol-crust enrichment factors (Table 1) for the elements

$$Ef_X(\text{Al, rock}) = (X/\text{Al})_{\text{aerosol}}/(X/\text{Al})_{\text{rock}}$$

showed similar trends of high values in the early samples which decreased smoothly to unity or lower with increasing haze aerosol. For V the trend was most marked; indeed of these elements V has the highest enrichments in cities (5–500 (ref. 3)). Taken together, the trends of these enrichment factors clearly indicated that the background aerosol was pollution-derived but that the haze aerosol itself was crustal, that is natural, just the opposite of what we had expected. Electron microscopy of the Nuclepore filters showed that angular crustal particles of diameter greater than roughly 0.4–0.8 μm are present in all samples, but with greatly increased numbers during the haze episode. In contrast, most particles smaller than 0.4–0.8 μm diameter were nearly spherical; their abundances were much less haze-dependent. Although we have studied only this one episode, we feel that it may well be typical, as discussed below.

The source of the crustal haze aerosol must be very strong to account for its high concentrations. It seems to be the great Takla Makan and Gobi deserts of eastern Asia. Upper-level constant-pressure charts indicate that our haze period was preceded by several days of intense air flow from these deserts to Alaska. Figure 1 shows the 700-mb isobaric trajectories for air arriving at Barrow before, during, and after the episode. Only episodic air had recently passed over the Asian deserts.

Several factors support the idea of an Asian source for Arctic haze. First, spring is the period of greatest dust storms in deserts. In our case, during nearly all of April 1976 the Asian deserts were filled with dust storms. Second, the composition of Arctic haze greatly resembles that of the Sahara aerosol (K. A. Rahn, L. Schütz, and R. Jaenicke, unpublished data). Third, flow patterns conducive to long-range transport from Asia to Alaska are strongest

Table 1 Elemental concentrations and enrichment factors for aircraft filter samples

Sample	AB	CD	E	F	G	IJ	KL	M	N
				Concentration [ng m^{-3} (ambient)]					
Al	10.0±0.9	17.0±1.0	35±2	34±2	91±5	240±10	203±10	240±10	14.7±1.7
V	0.035±0.006	0.148±0.010	0.126±0.012	0.094±0.010	0.22±0.02	0.40±0.03	0.37±0.02	0.40±0.04	0.0192±0.0100
Mn	0.146±0.012	0.36±0.02	0.59±0.04	1.58±0.03	1.55±0.08	3.0±0.2	3.1±0.2	3.4±0.2	0.21±0.02
Na	12±10	32±11	<30	21±16	47±27	58±15	37±11	37±30	<20
Ba	0.4±0.2	0.4±0.2	<0.8	<0.5	1.6±0.6	2.0±0.2	2.0±0.2	1.9±0.5	<0.5
				Enrichment factor (Al, rock)					
Al	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
V	2.1	5.2	2.2	1.66	1.46	1.00	1.10	1.00	0.79
Mn	1.25	1.81	1.44	1.46	1.46	1.07	1.31	1.21	1.22
Na	3.4	5.4	<2.5	1.77	1.48	0.69	0.52	0.44	<4
Ba	8	5	<4	<3	3.5	1.7	1.8	1.5	<6
Date	12, 13 April	14, 15 April	17 April	19 April	21 April	28, 29 April	30 April, 1 May	3 May	5 May
Volume (m^3)	249	341	149	176	110	309	365	88.7	108
Altitude (km)	3.3	2.0, 1.2	2.1	2.1	3.0	1.8, 2.3	2.1, 2.0	2.5 to 2.8	1.8

during the spring[4,5]. Fourth, the Asian deserts are farther north than the other deserts, lying mostly between 40° and 50° North as opposed to the more normal desert latitude of 20° to 30°. Fifth, the length of typical trajectories between Asia and Alaska (9,000–12,000 km) is not out of line with the well documented 6,000-km path for transport of Sahara dust to Barbados[6].

An Asian source for Arctic haze explains or confirms many previous observations such as:

(1) Anomalously high turbidities over Barrow, especially during the spring[7] (flow patterns between Asia and Alaska appear to be common from November through May);

(2) The large size of the haze bands (see below);

(3) Previous haze events at Barrow (for example, the episode of March–April 1974 was preceded by a few days of strong flow from the Asian deserts);

(4) Previous measurements of anomalous turbidities over Fairbanks, Alaska. For example, 17–22 February 1976 was a whitish haze episode in Fairbanks which affected air at all levels and for which no explanation could be found. Visibilities were reduced from the normal 150 km to less than 30 km. Trajectory analysis now reveals that this air was also of Asian origin. Similar haze incidents with trajectories leading back to Asia seem to be a regular feature of the Fairbanks atmosphere during winter and spring;

(5) The accumulation of brownish insoluble deposits in the pack ice north of Barrow, which under examination with a light microscope seem to be continental dust (R. Paquette, personal communication). The mineralogy and possible sources of this dust have also been discussed[8];

(6) Anomalous ice-nucleus concentrations at College, Alaska, Blue Glacier, Washington, and Nagoya, Japan during February and March 1968, for which trajectory analysis showed eastern Asia to be the probable source[9];

(7) A case of abnormally high condensation nucleus concentrations at Barrow in March 1970[10], which was explained as pollution from Prudhoe Bay to the east but which may have originated from Asia because the large-scale flow for this period was from those deserts.

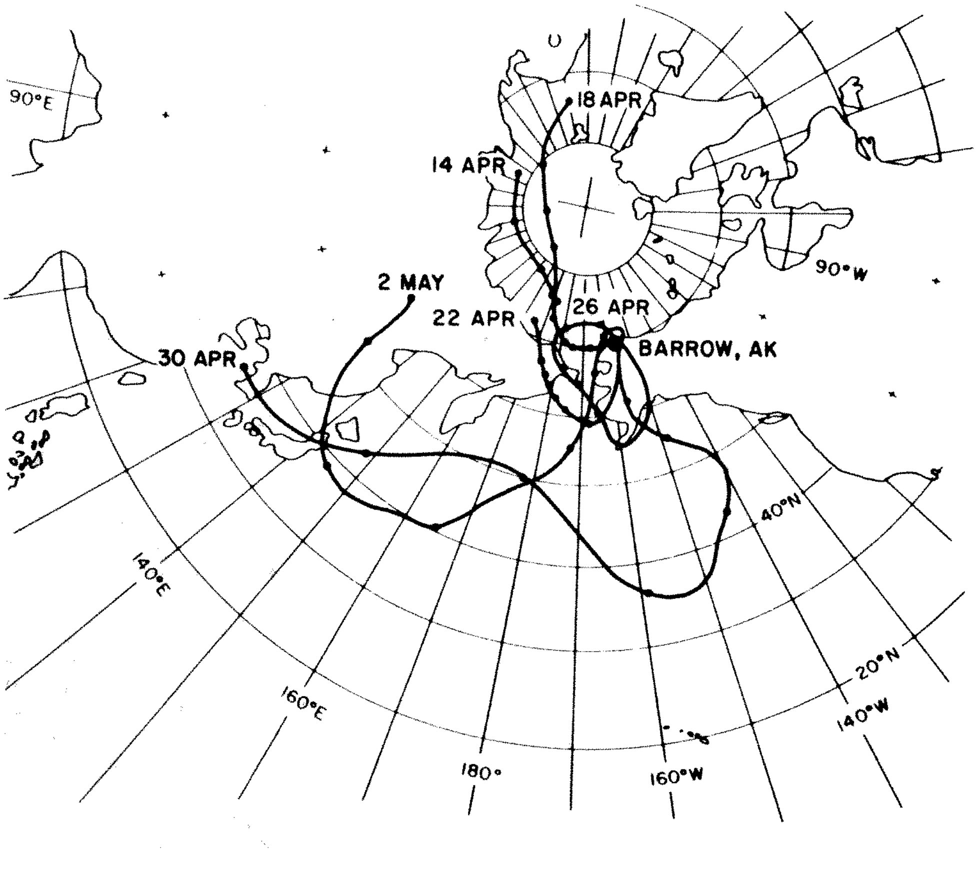

Fig. 1 700-mbar isobaric trajectories of air to Barrow. Numbers refer to date of arrival at Barrow. Solid circles each 24 hours along trajectory.

The mass of desert dust transported into the Arctic seems to be very great. For the five-day episode over Fairbanks discussed above, columnar mass loadings derived from radiation measurements showed values of about 45 mg m^{-2} (for aerosol density 2.5). For a point source in Asia and a dispersion angle of 5° (typical of volcanic plumes), the dust cloud would be 900 km wide at Alaska, or about the width of the state itself. (Indeed during this incident visibility was poor over the entire north half of the state.) Such a plume travelling at 80 km h^{-1} would carry 4,000 tons of dust into the Arctic per hour, or a half-million tons over the five-day episode. This is equivalent to skimming off a 0.2-μm layer from a 10° × 10° desert.

Clearly, more detailed studies are needed to refine our ideas about this significant phenomenon. Also, possible climatic effects of such large amounts of aerosol in the Arctic should not be overlooked.

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Sulphur dioxide discharge from Mount Etna

REMOTE sensing of SO_2 emitted by Mount Etna in its normal state of permanent activity, indicates much higher flow-rates than those previously measured by the same method (correlation spectrometry) on other volcanoes. A set of measurements in June 1975 provided a mean SO_2 flow-rate of 3,740 t d^{-1} for this volcano. This is a striking result both for volcanology and for environmental sciences. The knowledge of the mass discharge of chemical species from an active volcano and of its variations, both in time and space, is of great interest for the understanding of eruptive mechanisms and the establishment of geochemical balances. The SO_2 content of a volcanic plume can be measured from a distance by an optical correlation technique; this method was perfected a few years ago and has since been widely used for the remote sensing of air pollution in industrial areas[1]. During June 1975, we used the correlation spectrometer Barringer Cospec II to measure the SO_2 emitted by the active vents in the summit of Mount Etna (Central Crater, Bocca Nuova, North-East Crater and 1964 Crater), 3,200 m above sea level.

Activity within these craters was then characterised by a strong emission of condensed water vapour. From time to time, the plume issuing from the Central Crater was loaded with ashes produced by explosive activity taking place at least 500 m beneath the crater rim. At 2,600 m above sea level, on the northern flank of the volcano, the lateral eruption which had begun in February 1975 was still in progress, characterised by a slow effusion of an almost entirely degassed lava.

The remote sensing correlation spectrometer compares a high contrast reference spectrum (multiple slit mask) with the molecular absorption spectrum of the gas mixture examined (in the 3,100Å region for the sensing of SO_2). This correlation technique ensures the sensitivity and the selectivity of the instrument over a broad range of concentrations. The Cospec II displays the concentration times path length product of the SO_2 present along its optical path. In other words, the response of the spectrometer is directly proportional to the product p.p.m. (mean concentration of SO_2 in the sighted plume) × m (thickness of the plume) according to the Beer–Lambert's law. The skylight is the ultraviolet source for the spectrometer. Weak variations in the intensity of this source are automatically compensated; the stability of the instrument, in the absence of any cloud cover is maintained from three hours after sunrise until 3 h before sunset. Two quartz cells, containing the gas to be detected in two different concentrations, can be set on to the optical axis of the remote sensor: they are an internal calibration of the spectrometer. This internal calibration can be checked by sighting with the spectrometer just above the stack exit of a fossil fuelled power plant, whose SO_2 emission is accurately known (true scale external calibration).

On Mount Etna, measurements were made by the traverse method whereby the remote sensor equipped with its vertical sighting device is carried by car under the plume. A continuous westerly wind throughout our series of measurements provided the best meteorological conditions available on this site: the plume was drifting over a nearly straight motorway at sea level, 10 km downwind from the volcanic discharge area, on which the car could travel at constant speed between the cities of Giarre and Taormina. For each traverse, the signal of the spectrometer was recorded continuously. The SO_2 concentration of the plume appears on the strip chart as a nearly gaussian curve; its area is the product of concentration by surface (p.p.m. × m^2). From this measurement—corrected by taking into account the mean angle between the motorway and the plume axis —and from the mean wind velocity, one can deduce for each traverse the mass flow of the detected gas. The wind velocity was measured by teleanemometry. The teleanemometer used has been designed and constructed at Bradford University. It consists of an optical telescope and two photosensitive cells which measure the shifting rate of an illumination difference between two points of the field of the telescope. The signals of the two cells are treated on a digital correlator which can extract a mean delay time corresponding to the velocity of the sighted plume.

Table 1 displays the measurements from Mount Etna made during three days. The following conclusions can be drawn. When a main road exists near the base of a volcano, the traverse method ensures little dispersion of the measurements. Therefore, a systematic use of the optical correlation technique for the remote sensing of SO_2 could be used to detect slight variations of volcanic gaseous discharge that might indicate changes in eruptive activity.

Table 2 compares the mean values of SO_2 discharge from Mount Etna (17 traverses in 3 d) with those obtained from other volcanoes using the same technique. The flow measured from Mount Etna seems to be much higher than that measured from Central American or Japanese volcanoes, despite apparently similar activity (that is, moderate pyroclastic activiy) during the time of the measurements. Moreover, during violent pyroclastic activity, as emphasised

Table 1 SO_2 discharge from Mount Etna in June 1975

Date	Wind velocity ($km\ h^{-1}$)	Traverse number	Time (beginning of traverse)	SO_2 emission ($t\ d^{-1}$)
14 June 1975	30 (estimated)	2	11.00	4800
		3	11.30	3800
		4	12.30	3200
		5	15.00	2700
		6	15.30	3900
		7	16.00	3600
		9	16.30	4200
		10	17.00	3700
15 June 1974	36 (measured)	12	11.40	3300
		13	12.00	4200
		14	12.45	3800
		15	13.00	4000
18 June 1975	31 (measured)	1	11.00	4100
		2	11.35	3700
		3	12.20	3600
		4	14.25	3600
		5	15.00	3400

The normal permanent activity provides a very stationary SO_2 flow within the time scale used (duration of traverse under the volcanic plume: approximately 10min).

by previous authors correlation spectrometer measurements cannot be made because ash-laden plumes screen the skylight. For these two reasons, it seems hazardous to assume a global SO_2 discharge rate to the atmosphere for all cases.

SO_2 seems to be the main sulphur species in a volcanic gas plume emitted at high temperature and without a great amount of ash or condensed water vapour which could act as an oxidation catalyst and lead to the conversion of SO_2 into sulphates. This SO_2 prevalence is indicated by the generally low value of the SO_4^{2-}/SO_2 ratio obtained in volcanic gas plumes[2]. Measurements by Le Guern[3] on Mount Etna in June 1972 (activity similar to that of June 1975) showed that a sulphuric acid aerosol was emitted by the summit area of the volcano at a rate of 24 $t\ d^{-1}$. If as in fossil fuelled power plant fumes[4], SO_2 in volcanic discharge remains at this oxidation state for a long time as compared with the delay time between emission and observation, one can assume that the SO_2 flow accurately represents the total sulphur discharge of an Etna-like volcano. Hence, in order to measure the flow of all other chemical constituents of the volcanic gas phase, it is sufficient to measure their concentration in the plume against SO_2 concentration.

Nevertheless, the question of the oxidation rate of SO_2 in volcanic gas plumes during atmospheric diffusion is still unsettled. If this oxidation occurs more rapidly than mentioned above, then the flow rates reported in this paper are too low. In general, and essentially because of the light scattering by aerosols in the volcanic plume and in the atmosphere, all the SO_2 fluxes measured with the correlation spectrometer technique must be considered as minimums.

During our measurements of the SO_2 discharge from Mount Etna, the lava flow from the northern flank of the volcano had been estimated at $10^4\ t\ d^{-1}$. Assuming that 0.07% sulphur by weight is emitted by a basaltic magma while degassing[5], the estimated lava flow corresponds to a 14 $t\ d^{-1}$ SO_2 discharge from one of the summit craters of Mount Etna (in all likelihood the north-east one). This is far from the measured discharge and we may assume that the SO_2 originates from the degassing of a much greater mass of magma moving by convection within the volcano pipe. Using again the assumption of Moore and Fabbi[5], the mass flow of this convecting magma would at least represent $2.7\times10^6\ t\ d^{-1}$ ($\sim10^6\ m^3$). The more recent data[11] available on magmas more likely to reflect Etna's volatile content may suggest higher initial sulphur concentrations in the magma (up to 0.16% sulphur for basaltic liquids before effervescence). These data do not change the discrepancy between the observed lava effusion and the measured rate of outgassing.

Table 2 Comparison between SO_2 discharges from different volcanoes

Volcano	Date	SO_2 emission ($t\ d^{-1}$)	Ref.
O-Shima*	20 April 1971	345	6
Asama*	1 June 1972	142	7
Santiaguito†	1973	420	8
Fuego†	1973	40	8
Pacaya†	1973	260	8
San Cristobal‡	1973	360	8
Telica‡	1973	20	8
Momotombo‡	1973	50	8
Masaya‡	1973	180	8
Asama*	28 April 1974	787	9
Kilauea§	9–19 Feb. 1975	280	10
Salfur Banks§	1975	7	10
Mauna Ulu§	1975	30	10
Etna	14, 15, 18 June 1975	3740	This paper

In all cases, the same sensing technique was used.
* Japan.
† Guatemala.
‡ Nicaragua.
§ Hawaii.

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Superheavy element fission hypothesis in carbonaceous chondrites

IN many meteorites, the abundances of the heavy isotopes of Xe are enriched relative to solar Xe. In some cases, this enrichment results from the addition of fission products from U and Pu. In some carbonaceous chondrites, however, the isotopic enrichment pattern of the heavy Xe is inconsistent with the measured Xe yield patterns of U, Pu, Cm, and Cf fission. The lack of correlation between heavy Xe enrichment and U concentration in separated mineral phases also argues that the Xe is not due to fission of an element whose chemical properties are similar to U. It has been suggested that this enrichment resulted from the decay of a superheavy element[1,2]. An experimental test of this hypothesis is proposed here.

Lewis *et al.* have determined that most of the trapped Xe in Allende and several other carbonaceous chondrites is contained in chromite, carbon and an unidentified phase, *Q*, which together constitute less than 0.5% of the total meteorite mass[3,4]. One component of this Xe is enriched in the heavy Xe isotopes, and Anders *et al.* consider this to be evidence for the decay of a now extinct, fissioning superheavy element associated with these minor phases[5].

In favourable circumstances, nuclear tracks produced by fission decay can be revealed by chemical etching or transmission electron microscopy. Flynn *et al.* have searched for tracks in chromite grains from Allende, and found none. They placed an upper limit on the *in situ* fission which is two orders of magnitude below that implied by the superheavy hypothesis[6]. Macdougall *et al.* examined an olivine adjacent to chromite in Murchison, selected from a separate which also contains the heavy Xe enrichment, and found no fission tracks[7]. These track studies, however, are inconclusive, since tracks in chromite and olivine might have been removed by a thermal event which would not have removed the Xe.

A more conclusive test of the superheavy fission hypothesis would be a search for fission related isotopic anomalies in other elements. These anomalies could be less affected by thermal events than the Xe anomaly or fission tracks. Furthermore, isotopic measurements could also be made on the carbon and *Q* phases, which are not suitable for track studies.

In samples of bulk meteorites, the fission contributions to elements other than Xe and Kr are too small to be detected because of the greater natural abundances of the other elements in solid materials. If, however, the extreme enrichment of heavy Xe in these minor phases of meteorites is due to fission there should be corresponding enrichments of other fission products.

The light rare earth elements are particularly well suited for a search for these isotopic enrichments because: (1) the natural abundances of rare earths are low; (2) the fission yields of rare earths are high; (3) the techniques for accurate mass spectrometric measurement of rare earth isotopic ratios have recently been developed, and (4) the constancy of rare earth isotopic ratios, except where altered by nuclear processes, has been established.

The fission decay properties of U and the transuranic elements help predict which elements should exhibit the largest isotopic anomalies from the possible superheavy decay. The mass yield data for spontaneous or thermal neutron fission of ^{235}U, ^{233}U, ^{239}Pu, ^{242}Cm, and ^{252}Cf all show a double peaked distribution with two rather broad maxima. The principal effect of an increase in mass of the fissioning nucleus is to shift the light mass peak to higher values, while the heavier mass peak remains essentially unchanged[8]. This behaviour, shown in Fig. 1, is explained by 50 proton and 82 neutron closed shell effects for nuclei on the heavy mass peak.

The heavy Xe yield data for Allende minor phases[9], when normalised to ^{136}Xe = 6%, fits well on the left side of the high mass peak (Fig. 1). This suggests that if the heavy Xe is of fission origin the relative yields of Ba and the light rare earths, which are nearby in mass, should be of the same magnitude as for the thermal neutron fission of ^{235}U, which has been well studied.

Using ^{235}U mass yields, we can calculate the quantity of each rare earth and Ba isotope which would be produced by the amount of fission required to give the observed heavy

Fig. 1 Mass yield curves for thermal-neutron fission of ^{233}U (——), ^{235}U (– – – –), ^{240}Pu (–·–·), and spontaneous fission of ^{252}Cf (····) (figure from ref. 17). ■, Yields of ^{136}Xe attributed to fission from the Allende Xe rich phases, normalised to ^{136}Xe = 6% yield (data from ref. 3).

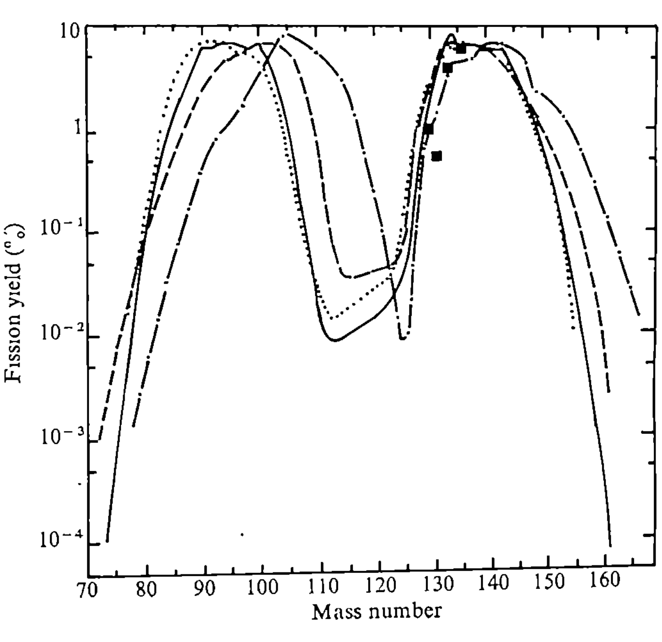

Table 1 Estimated fission induced deviations from normal isotopic ratios in the Allende Xe-rich phases

Isotope	Fission product*	δ(‰) Q	δ(‰) chromite	δ(‰) carbon
^{130}Ba	no	0	0	0
^{132}Ba	no	0	0	0
^{134}Ba	no	0	0	0
^{135}Ba	yes	+1.3	+0.25	+0.36
^{136}Ba	no		(referenced isotope)	
^{137}Ba	yes	+0.73	+0.14	+0.20
^{138}Ba	yes	+0.11	+0.02	+0.03
^{138}La	no	−0.73	−0.14	−0.20
^{139}La	yes		(referenced isotope)	
^{136}Ce	no	−2.2	−0.44	−0.62
^{138}Ce	no	−2.2	−0.43	−0.61
^{140}Ce	yes	−1.9	−0.35	−0.51
^{142}Ce	yes		(referenced isotope)	
^{142}Nd	no	−1.3	−0.26	−0.40
^{143}Nd	yes	+1.5	+0.28	+0.39
^{144}Nd	yes		(referenced isotope)	
^{145}Nd	yes	+1.4	+0.25	+0.34
^{146}Nd	yes	−0.30	−0.07	−0.09
^{148}Nd	yes	+0.35	+0.08	+0.12
^{150}Nd	yes	−0.66	−0.14	−0.22
^{144}Sm	no	0	0	0
^{147}Sm	yes	+2.5	+0.48	+0.67
^{148}Sm	no		(referenced isotope)	
^{149}Sm	yes	+1.3	+0.24	+0.34
^{150}Sm	no	0	0	0
^{152}Sm	yes	+0.16	+0.04	+0.06
^{154}Sm	yes	+0.16	+0.02	+0.02

*This column indicates whether fission contributes to the isotope in question. The estimated isotopic enrichments and depletions are based on the assumptions that the yield curve is the same as for ^{235}U thermal neutron fission[8], the natural abundances of the elements are average carbonaceous chondrite abundances[15], and the natural isotopic ratios are terrestrial[16]. The quantities of fissiogenic ^{136}Xe in the Q, chromite, and carbon phases of Allende were taken as 3.7, 0.7, and 1.0×10^{-8} cm^3 STP g^{-1} (ref. 4).

Xe anomaly. Since the abundances of Ba and the rare earths in chromite, carbon, and Q from Allende have not been well established, we assume these abundances to be average chondritic and the natural isotopic ratios to be terrestrial. The deviations of the isotopic ratios from the terrestrial ratios due to the addition of fission products can then be estimated (Table 1). Several of these deviations are within the limits of detection reported by groups measuring the rare earths[9,10] and Ba[11].

Measurement of isotopic anomalies in these elements would confirm the fission origin of the heavy Xe. Should the progenitor be a known transuranic element, and the anomalous Xe some form of fractionated fission Xe, it might be possible to match the rare earth enrichments to the measured yields for a transuranic.

Should the progenitor be a superheavy, we might expect some broadening of the heavy mass peak, as seen for ^{252}Cf (Fig. 1), which would increase the yield of heavy rare earths. The yield of ^{156}Gd, for example, is 50 times greater from ^{252}Cf fission than from ^{235}U fision. Even larger amounts of ^{156}Gd might be expected from superheavy fission. Measurement of large isotopic anomalies in the heavy rare earths would suggest the progenitor was a superheavy. The absence of large isotopic anomalies in the heavy rare earths, however, would not eliminate the possibility of a superheavy progenitor. Ternary fission, which might not produce the heavy rare earths, has been suggested as a possible mode of fission decay for superheavy elements[12].

Other nuclear effects which might contribute to changes in the isotopic ratios are fission products from U and Pu, neutron capture production, and spallation. A U abundance of 100 p.p.b. has been measured in the heavy Xe-rich Allende separate (J. Shirck, personal communication), while Anders *et al.* have measured 3 p.p.b. in a chromite–spinel fraction of the Xe-rich separate[5]. Even using the larger 100 p.p.b. value and a Pu/U ratio of 0.016 at formation[13], the total contribution of U and Pu fission products over the 4.5×10^9 yr age of the meteorite is two orders of magnitude below the estimated superheavy contribution.

Neutron capture effects can be estimated from the ^{150}Sm/^{149}Sm ratio. Fission shielded ^{150}Sm is produced by a (n,γ) reaction on ^{149}Sm, which has the largest cross section of any Sm isotope. An upper limit on the neutron flux can already be set from the ^{80}Kr and ^{129}Xe measurements of Lewis *et al.*[3].

Isotopic contributions from spallation can be estimated from the proton flux, derived from spallation contributions to Xe and Kr, and measurements of the abundances of the various spallation targets.

The actual abundances of the rare earths and Ba in chromite, carbon, and Q have not yet been well established. However, Anders *et al.* report abundances for several rare earth elements in an Allende chromite separate to be depleted by about a factor of 5 from chondritic[5]. This would increase the deviations from natural isotopic ratios in the chromite phase by a factor of 5 from those given in Table 1.

Despite the elusiveness of Q, it might prove to be the most suitable phase for isotopic measurements since: it is most enriched in the anomalous heavy Xe, and it is soluble in nitric acid, providing the means for separation of Q and its associated rare earths from the chromite and carbon phases.

The technique of measuring rare earth isotopic enrichment patterns has already proved valuable in identifying the ^{235}U thermal neutron fission products in samples from the Oklo natural reactor[14]. Measurement of Ba and rare earth isotopic anomalies associated with the heavy Xe anomaly in carbonaceous chondrites could finally establish the fission origin of this heavy Xe. This technique would also be applicable to other samples where large concentrations of superheavy fission are expected.

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An early Cambrian chert biota and its implications

In the search for Precambrian life, much effort has been centred on the study of stromatolitic cherts and their predominantly blue-green algal flora[1]. Nonetheless, it is common for reviews of evolution of the biosphere to switch from consideration of Pre-Vendian chert floras to Vendian soft bodied or trace fossils and thence to Tommotian shelled invertebrates[2]. A more objective approach will involve tracing organic development across the Cryptozoic–Phanerozoic transition from within a single habitat. Until this has been attempted, our views of changing biotas will be obscured by the changing facies patterns. I report here the discovery of a new early Cambrian flora and fauna from a typically 'Precambrian' habitat, a chertified oncolite association.

The chert horizon occurs within parallel laminated dolomites about 10 m above the base of the Eilean Dubh Formation of the Durness Group of north-west Scotland, near to Inchnadamph (NC 253213). Except for an unverified report of *Salterella*[3] this is the first report of organisms from this predominantly dolomitic formation and confirms the early Cambrian age of the lower part. The underlying strata contain shelly fossils typical of Lower Cambrian rocks while overlying horizons within the Durness Group are of early Ordovician age[3].

The complex diagenetic history of these Durness dolomites[4] is evident from the varying degrees of organic and skeletal preservation in the Inchnadamph chert. Invertebrate skeletal remains, preserved in silicified dolomite clasts, have suffered much recrystallisation before silicification. Preliminary identifications of rare fragments show the presence of trilobites, hyolithids (Fig. 1*a*), sponge spicules and archaeocyathids (Ajacicyathacea). The presence of *in situ* invertebrates is indicated by pellet-filled burrows in the associated laminated dolomites.

Algal participation in carbonate accretion is shown by the abundant filamentous and coccoid structures preserved as kerogenous or pyritic material in ooliths and oncoliths (Fig. 1). Unbranched, septate filaments of 2–5 μm diameter occur as envelopes around oolith laminae and in the outer layers of oncoliths, mostly lying normal to the surface (Fig. 1*a, f*). These closely resemble recent and fossil filamentous blue-green algae from similar facies[5,6] and the mucilage probably contributed to oolith growth.

Branched filaments 1–3 μm in diameter occur as small, isolated and erect tufts up to 25 μm across, preserved in dark pyritic material (Fig. 1*b, d*). Their form and oncolithic habit compare closely with *Corbularia conglutinata* Vologdin from the Lower Cambrian of Asia[7]. The small cell diameter suggests cyanophyte affinities.

The problematic organism *Renalcis* is excellently preserved in several oncoliths, revealing forms comparable with *R. levis* Vologdin (Fig. 1*c*). This material is clearly not foraminiferid and resembles colonies of coccoid cyanophytes embedded in a gelatinous envelope (compare with *Anacystis*). I have previously argued in conjunction with Riding[8] that the 'chamber walls' of *Renalcis* cannot represent the single cell wall of blue-green alga, and here they are seen to consist of a chain of minute coccoid cells about 2 μm in diameter, their packing reduced towards the central 'chamber'. This, and evidence of shrinkage cracks in other material, leads one to support the blue-green algal interpretations of Korde[9] and Hofmann[10]. Nevertheless it is possible that *Renalcis* is an artificial and polygot genus, encompassing other algal and protistan groups.

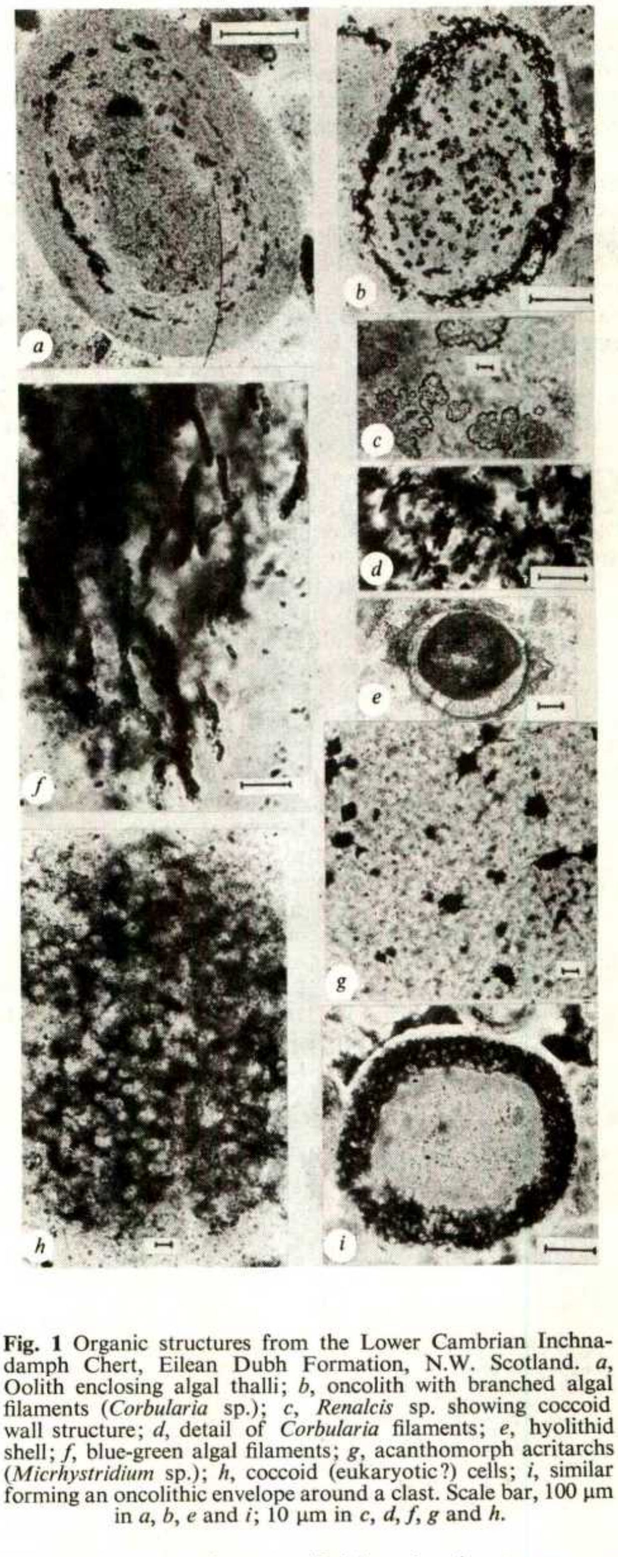

Fig. 1 Organic structures from the Lower Cambrian Inchnadamph Chert, Eilean Dubh Formation, N.W. Scotland. *a*, Oolith enclosing algal thalli; *b*, oncolith with branched algal filaments (*Corbularia* sp.); *c*, *Renalcis* sp. showing coccoid wall structure; *d*, detail of *Corbularia* filaments; *e*, hyolithid shell; *f*, blue-green algal filaments; *g*, acanthomorph acritarchs (*Micrhystridium* sp.); *h*, coccoid (eukaryotic?) cells; *i*, similar forming an oncolithic envelope around a clast. Scale bar, 100 μm in *a*, *b*, *e* and *i*; 10 μm in *c*, *d*, *f*, *g* and *h*.

Larger coccoid cells 12–25 μm in diameter are probably eukaryotic (Chlorophyta?). These occur singly or as colonies coating the outer margins of round carbonate clasts (Fig. 1*h, i*) and occur throughout some oncoliths. Cell walls and contents are not preserved, their form merely being outlined by strongly pigmented material that was once a mucilaginous matrix (compare ref. 10). Acanthomorph acritarchs 12–20 μm in diameter (*Micrhystridium* sp.) are evenly distributed through a few silicified dolomite clasts (Fig. 1*g*) where they are associated with archaeocyathid fragments.

The scarce invertebrate remains, acritarchs and *Renalcis* suggest that sediments were deposited in an offshore facies, perhaps like that of the Forteau Formation of Newfoundland with which this horizon could be correlated. The oncolithic flora clearly shares with its Precambrian precursors an inferred abundance of blue-green algae but differs in the predominance of tufted forms such as *Corbularia*. While the growth forms of *Renalcis* can be matched with coccoid cyanophytes from the Precambrian[10], their expansion in biohermal facies was clearly a Phanerozoic, Tommotian event[7] as was the radiation of spiny acritarchs[11]. Comparisons with similar late Precambrian biotas indicate that innovations were taking place in the Cambrian oncolite habitat. Invertebrates had begun to graze and burrow extensively, as predicted by Garrett[12]. Coccoid eukaryotes had developed the oncolithic mode of growth. Even the ultraconservative prokaryotes show a radiation of oncolithic forms, including skeletal oncoliths like *Girvanella*[13], and biohermal forms like *Renalcis*.

Evidently the 'Cambrian evolutionary explosion' was not confined to metazoans, to eukaryotes or to skeletal forms. Old 'Precambrian' niches remained but were modified. The simplest explanation for these wide-ranging changes is an ecological one, based on the cropping principle[14,15]. Increased grazing pressure by microphagous benthic organisms brought not only a general diversity increase but also the associated phenomena of skeletalisation, size increase and burrowing. Skeletalisation is seen, amongst the present biota, in the spiny cuticularised wall of the acritarchs, which may have served to resist digestion by deposit feeders. Permineralisation and size increase in early Cambrian algae would have performed a similar function, to discourage overcropping by herbivores. The disruption of stromatolite growth by burrowers is also seen and must have favoured the development of unattached oncolithic algae like *Corbularia* and *Girvanella*. Further searches may reveal the soft tissues of invertebrates of the same age preserved in this or similar cherts.

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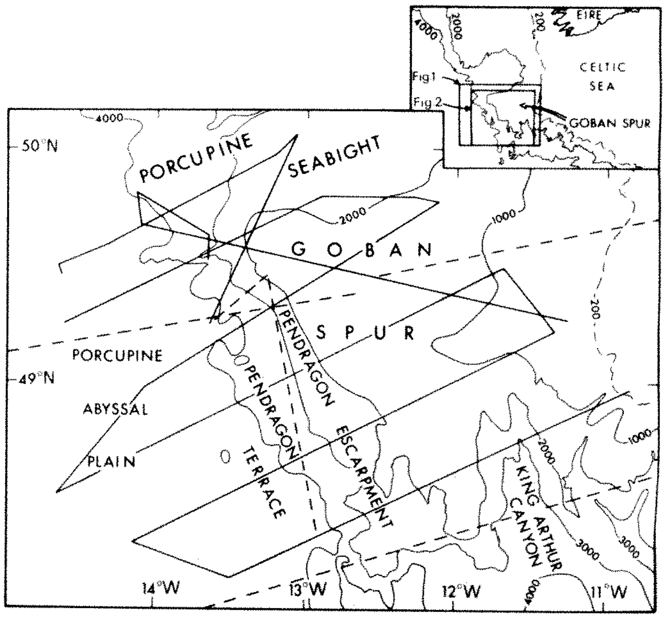
Continental margin fault pattern mapped south-west of Ireland

A SIMPLE model for continental basement structures at rifted continental margins comprises large fault blocks which trend approximately parallel to, and step down towards, the continental–ocean boundary (for example, see ref. 1). These blocks may be cut by faults which strike across the margin, and, in many theoretical discussions, are shown as being separated from the true oceanic crust by an intermediate zone (see transitional crust of Fig. 3, ref. 2). On many rifted margins these features are deeply buried by young sediments and cannot be stutied in detail. On Goban Spur (Fig. 1), a marginal plateau south-west of Ireland, the young sediment cover is abnormally thin, however, and we have been able to map in detail a 150 km wide continental basement fracture pattern of horsts and grabens using a simple seismic reflection system (160 inch^3 air-gun and two-channel hydrophone array). We also suggest a location for the continent–ocean boundary between the Spur and Porcupine Abyssal Plain. There are few previously published data from Goban Spur relevant to our study, although valuable sampling[3] and geophysical[3–5] results have been obtained north and south of the area.

The results of our surveys (Fig. 1) indicate a threefold subdivision of the continental margin from east to west: block-faulted continental basement (Goban Spur); intermediate zone (Pendragon Terrace); and oceanic basement (Porcupine Abyssal Plain) (Figs 2 and 3). North of 49°N the margin is cut by at least two large fault zones (fault zones 1 and 2), to the south of which the threefold subdivision is most easily recognised. Beneath the central and southern parts of Goban Spur, there are several large, north-west–south-east trending basement highs. These are most probably fault bounded, and are separated by basins containing relatively thick sedimentary sequences (>1.5 s double time). The amplitude of the basement discontinuities between high and basin is generally between 1 and 2 s double time, with the mean depth to basement decreasing from west to east. Alhough there is some evidence for rotaion of the fault blocks (for example, at 49°00′N, 12°40′W), our data best fit a model of ridges or horsts and grabens. Sample evidence indicates that the basement highs and, probably, the whole basement beneath Goban Spur, is continental in composition (granitic, metamorphic and old sedimentary rocks—ref. 3 and our unpublished data).

Goban Spur is separated from the continental shelf and the Pendragon Terrace by large boundary discontinuities, presumed to be faults (inner boundary fault and Pendragon Discontinuity respectively). The amplitudes of these dislocations are about 2.0 and 1.5 s double time respectively, and although the latter is not particularly large it does coincide with a change in basement characteristics. Our

Fig. 1 Track chart of seismic lines occupied by RRS Shackleton over Goban Spur during cruises 5/76 and 4/77 (- - - -, bathymetry only). Bathymetry in m after various sources. Inset shows location of Figs 1 and 2.



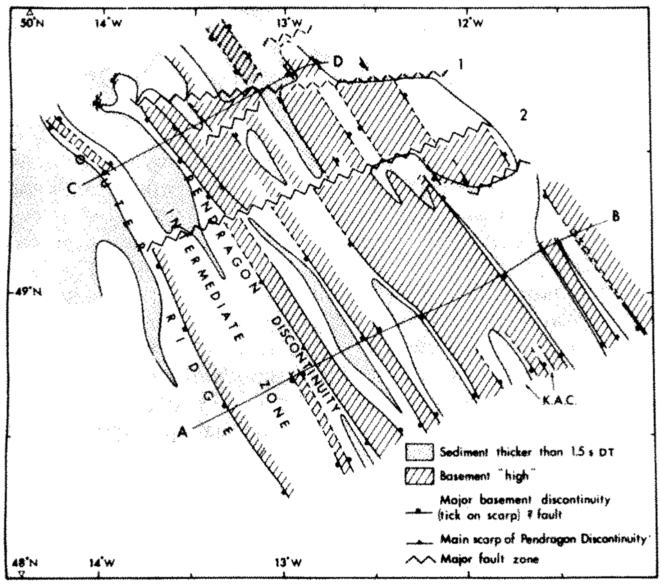


Fig. 2 Summary structural map of Goban Spur and adjacent areas, K.A.C., King Arthur Canyon. Sections AB and CD are shown in Fig. 3.

gravity data indicate that it also marks the point of maximum change in thickness of the crust. Where the Pendragon Discontinuity acts as a sediment dam it underlies a prominent bathymetric feature called Pendragon Escarpment, but in the south, the escarpment lies about 10 km west, over a subsidiary ridge.

The Pendragon Discontinuity separates Goban Spur proper from the intermediate zone. Here, basement morphology is relatively subdued (0.5 s double time in amplitude), and generally lies about 1.0 s double time deeper than to the east. The basement of the intermediate zone south of fault zone 2 is covered by a relatively thin sedimentary sequence, and although we have no sample evidence for its composition, geomagnetic characteristics suggest that it is largely similar to that beneath the spur. A large magnetic anomaly occurs, however, over the outer part of Pendragon Terrace, indicating the presence of basic igneous rocks. An outer ridge (Fig. 2) separates the intermediate zone from oceanic crust under the Porcupine Abyssal Plain, where basement morphology is rough but of low amplitude. The depth to oceanic basement is about 7.0 s double time and the sediment cover is relatively thin. Linear magnetic anomalies resulting from regular seafloor spreading are not well developed until 14°30′W is reached, although the magnetic field is rough and indicative of oceanic basement. We tentatively identify the western flank of the outer ridge as the location of the continent–ocean boundary.

Having briefly described the tripartite division of the margin structure where it is most clearly developed in the south, we now consider the northern part of Goban Spur, where the fracture pattern is crossed by fault zones 1 and 2 (Fig. 2). Fault zone 2 is a complex structure. Beneath Goban Spur it seems to act as a scissor fault, throwing the crest of the Pendragon Discontinuity up to the north, but the general level of basement to the east of the discontinuity down to the north. The fault also causes an alteration in the strike of the Pendragon Discontinuity (making it more westerly), coincides with an increase in amplitude of the discontinuity to about 4.0 s double time, and displaces it slightly in a dextral sense. Beneath Pendragon Terrace, however, zone 2 appears to act as a simple fault throwing down to the north. The depth to basement in the intermediate zone increases from about 5.5 s to about 7.3 s double time across the fault. Evidence that fault zone 2 has influenced the development of oceanic basement under the nearby parts of Porcupine Abyssal Plain comes from magnetic anomaly data, which show that sea-floor spreading anomalies are better developed to the north of the fault. An increase in sediment thickness in the abyssal plain is also observed across the westward extension of fault zone 2. Despite these complexities we can confidently recognise the tripartite subdivision of the continental margin between fault zones 1 and 2.

Fault zone 1 constitutes the northern structural boundary of Goban Spur. To the north of this, basement highs and lows are down-faulted into the southern part of Porcupine Seabight, so that the general level of basement is 2–3 s double time deeper. The thickness of the sedimentary sequence increases rapidly across the fault zone. Although the general structural pattern of Goban Spur can be traced into Porcupine Seabight, it is not possible to follow individual features, such as the Pendragon Discontinuity, and hence we do not know if there is any strike-slip component of motion on fault zone 1. The horst and graben style of tectonics identified beneath Porcupine Seabight has approximately the same trend as that beneath Goban Spur and may owe its existence to the same phase of rifting.

We believe that we have mapped a continental margin fracture pattern some 150 km wide that was initiated during the rifting phase that preceded the opening of the North Atlantic Ocean at this latitude. The trend of the basement horsts and grabens is parallel to the seafloor spreading magnetic anomalies observed further west[6]. On the evidence of these anomalies, the opening of the North Atlantic hereabouts is thought to have begun in Upper Cretaceous times. This would suggest that the rifting phase is of Cretaceous age. Our records show that there has been little or no vertical movement between adjacent fault blocks since the rifting phase. Thus, the subsequent history of the margin is one of overall limited subsidence. There is good evidence for Goban Spur being underlain by Hercynian granites[3,7], whose relative buoyancy in the crust could account for the lack of subsidence and thin sediment cover. Fault zones 1 and 2 are approximately parallel to the Hercynian structural grain projected from areas surrounding the Celtic Sea. Apparently, the rifting tectonics have been modified by reactivation of these old trends to produce the observed fracture pattern.

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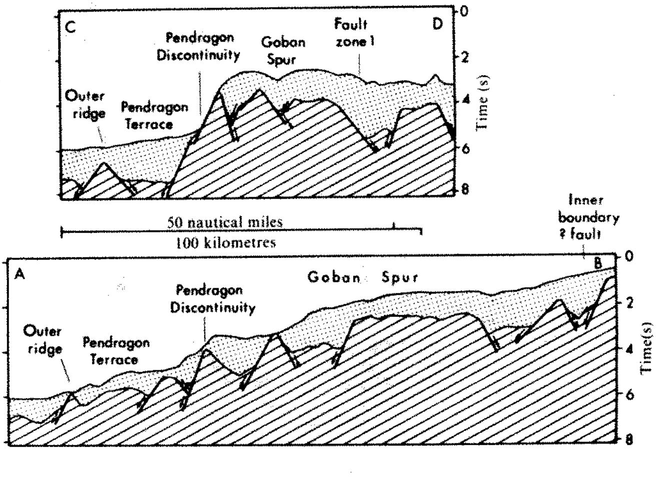
Fig. 3 Cross sections across Goban Spur replotted from seismic reflection profiles. Vertical scale is in seconds double time. See Fig. 2 for locations.



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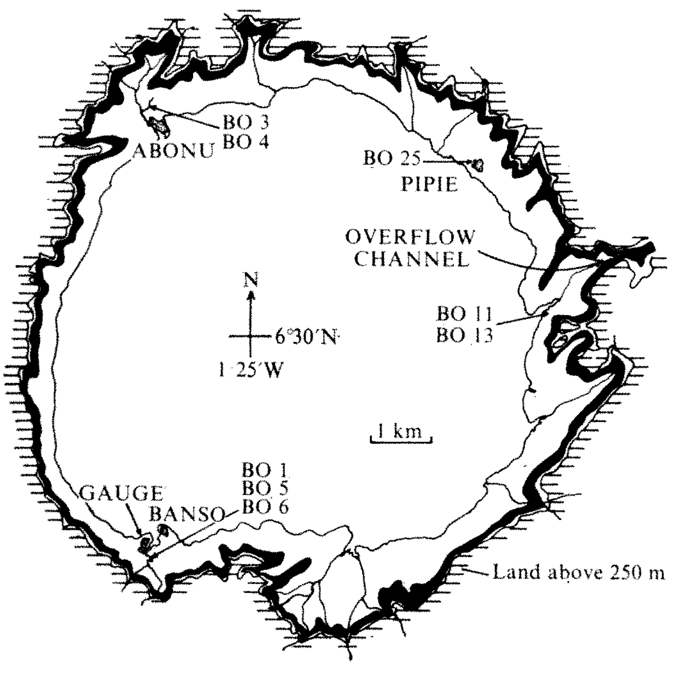
Holocene variations in the level of Lake Bosumtwi, Ghana

RECENT surveys of the changing climate of Africa during the Late Quaternary have shown that although a reasonably well established climatic sequence is now available for some parts of the continent, there exist very large areas which have yet to yield any well-dated, unequivocal palaeoclimatic data[1–3]. Most notable among the latter are the lowland tropical forest regions. Despite the absence of information from such critical areas, attempts have been made to reconstruct atmospheric conditions over West Africa during the Late Quaternary[2,4,5], but obviously no uniformly satisfactory models can be produced until these data gaps have been filled. Study of changing lake levels has proved a particularly valuable palaeoclimatic tool[3] and we report here the first ^{14}C dates of former levels of a lake that lies within the present lowland forest zone of West Africa. Our work indicates that lakes of the humid tropics can provide valuable palaeoclimatological information that may be less apparent from the study of arid zone lakes. In particular, we present evidence suggesting that major regressions of equatorial African lakes during the Holocene, rather than being a consequence of exceptional aridity, may have occured during periods of slightly higher temperatures and greater evaporation.

Fig. 1 Map of Lake Bosumtwi and inner part of crater, showing location of samples detailed in Table 1. Solid band below 250 m is erosional bench at level of overflow channel.



Lake Bosumtwi lies within a steep-sided crater that was produced by meteoritic impact 1.3±0.3 Myr ago[6]. The lake has a maximum depth of about 80 m, and in 1976 its surface lay at +100 m OD, some 105 m below the lowest point in the surrounding rim of hills and 100–200 m lower than the land outside the crater. Maintenance of the lake is thus largely dependent upon rainfall over the crater, a catchment of about 400 km^2. Mean annual precipitation is 1,520 mm and temperature 26 °C.

A clearly defined wave-cut bench encircles the lake at about 110 m above present lake level and merges into the lower slopes of the valley that provides the lowest point of exit from the crater (Fig. 1). This, the only valley to cut cleanly through the crater rim, has obviously functioned as an overflow channel in the past. No material suitable for dating has been found here, nor on the bench, all the dated samples coming from lacustrine sediments exposed within +25 m of the present lake surface.

Sample sites are shown on Fig. 1; samples and their ^{14}C ages are summarised in Table 1. On the basis of these results, plus historical records[7,8] and data from the Geological Survey Department's gauge at Apewu (1933 onwards), an attempt has been made to reconstruct the behaviour of Lake Bosumtwi over the past 10,000 yr (Fig. 2). Sediments older than the turbidite silts (Table 1) have not been identified[9]. This is somewhat surprising in view of the relative antiquity of the lake, particularly as the strong development of the high bench and overflow channel testify to one or more prolonged periods of maximum lake level.

The Holocene behaviour of Lake Bosumtwi shows some similarities with lakes elsewhere in West and East Africa. Generally high levels are known to have occurred throughout the tropics during the early Holocene[2,3]. In the case of lakes that occupied basins in what are now arid or semi-arid regions, the climate must have been markedly wetter then than now. Even though Bosumtwi was also high at this time it cannot be demonstrated that precipitation here was necessarily greater than at present. The lake has been rising continuously for at least the past 120 yr (Fig. 2), so prevailing climatic conditions seem adequate to maintain a very high level. Indeed, if evaporation from the lake surface is taken to be about 1.45 m yr^{-1} (ref. 10) (mean annual pan evaporation at Kumasi, 30 km away, was 1.4 m over the period 1954–1974), then a simple mass balance calculation is sufficient to show that the present hydrological regime is capable of sustaining the lake at overflow level. It is thus possible that around 9,000–10,000 yr BP the climate was essentially similar to today. Abundant leaf impressions preserved in the turbidites indicate that although the forest flora may have differed slightly in composition from modern local forests, the climate of the early Holocene must have been comparable with the present[11].

Unfortunately, we have been unable to obtain dateable material from the upper part of the turbidite silts and so have no precise idea how long the very high stand persisted. The considerable thickness (~ 35m) of silts above the dated horizons at Banso suggests that it continued well into the Holocene. The regression that followed clearly involved a considerable fall in lake level, the resulting erosion surface having been identified at elevations ranging from +2 m to +18 m. The subsequent transgression was coeval with positive changes in level that are known to have occurred in lakes throughout tropical Africa around 2,000 yr BP–3,000 yr BP (refs 2 and 3).

Table 1 Summary of samples* with ^{14}C ages

Sample no. locality	Lab. no.	Nature of sample	Geological context	State of lake	^{14}C age (yr b.p.)
B01 Banso	GIF—3650	Wood	Turbidite silts	Very high > +35 m	9880±220
B05 Banso	GIF—3991	Wood	Turbidite silts	Wood accumulated in deep anoxic waters of a stratified lake	10000±220
B03 Abonu	GIF—3651	*M. tuberculata*	Deltaic sands	Moderately high ~ +23 m	2520±200
B04 Abonu	GIF—3652	*M. tuberculata*	Deltaic sands	Moderately high ~ +25 m	1940±300
B06 Banso	GIF—3992	Wood	Root penetrating turbidite silts	Rising, vegetation killed by transgression	3620±110
B013 Konkoma	GIF—3996	Wood	Remnants of vegetation that colonised exposed turbidite silts		3020±110
B011 Konkoma	GIF—3995	*M. tuberculata*	Deltaic sands	Moderately high ~ +18 m	700± 90
B025 Pipie	GIF—3998	*M. tuberculata*	Silt between granite boulders	Moderately high > +19 m	1650±110

*Used in preparation of pre-nineteenth century section of Fig. 2. For precise sample localities see Fig.1.

Historical records[7,8] and the oral traditions of fishermen living around the lake are consistent in indicating that 200–300 yr ago Bosumtwi was very low indeed. The geological evidence for moderately high levels persisting until around 700±90 yr BP implies, therefore, that a rather abrupt regression must have started in the thirteenth or fourteenth century. In attempting to understand the regression of Bosumtwi, it is helpful to compare the behaviour of the lake with trends established for other climatic zones in West Africa, comparative palaeoclimatic data from elsewhere in the forest zone being nonexistent. Over the period of systematic observation, variations in the rate of rise of Bosumtwi have closely matched variations in the precipitation of the Sahel[12] (Fig. 3). Although climatic oscillations in the two regions may not always have progressed in unison (ref. 13 and J. Maley personal communication), the Sahel can provide a particularly sensitive indication of changes in the precipitation brought by the monsoon. Figure 3 shows, for example, that the Sahelian drought of the late 1960s and early 1970s was clearly reflected in Lake Bosumtwi by a period of lower than average rises. The lake, however, continued to rise, showing that this region of the forest was not exposed to true drought. Obviously here regression is only to be expected when monsoonal precipitation is drastically reduced and the Sahel subject to prolonged and exceptional aridity. This observation is of considerable importance in understanding the behaviour of Lake Bosumtwi in the more distant past.

Looking first at the period between 700 yr BP and the present, there is evidence to suggest that during the seventeenth and eighteenth centuries parts of the Sahel were slightly moister than at present[14], while there was at least abundant rainfall in the Chari–Logone catchment with which to maintain Lake Chad at very high levels[13]. At the same time, by analogy with the twentieth century, Lake Bosumtwi should have been very high or rising rapidly. The latter would certainly be consistent with oral traditions, which although indicating low lake levels, also abound with references to lake shore villages being abandoned because of inundation[7,8]. Thus the last regression must have occurred between the thirteenth and sixteenth centuries.

Over the last 1,000 yr Lake Chad is known to have oscillated considerably in level. A number of regressions are recognisable, including one which started between the twelfth and thirteenth century[4,13] and may, therefore, have been synchronous with the last fall of Lake Bosumtwi. None of these low stands of Lake Chad, however, seem to have been of long duration, the latest accompanying the recent drought and the last three occurring within the past 150–175 yr (ref. 15). Throughout this period, Bosumtwi has risen continuously, so monsoonal precipitation in the forest zone can clearly never have been greatly reduced during the nineteenth and twentieth centuries, despite decreases on a scale sufficient to allow Lake Chad to fall. As the twelfth to thirteenth century regression of Lake Chad was of apparently comparable magnitude with these more recent

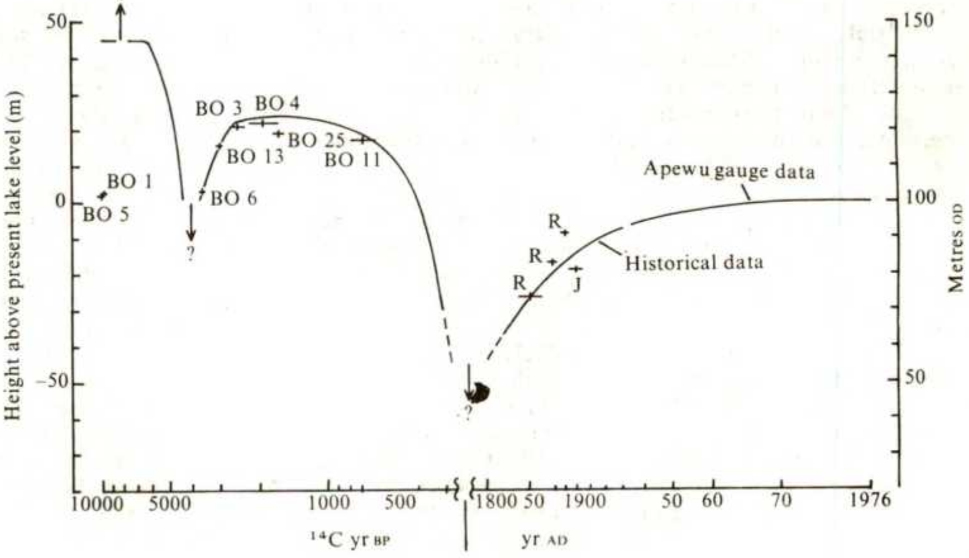


Fig. 2 Holocene fluctuations in level of Lake Bosumtwi (BO). Curve constructed from: (1) ^{14}C dates of samples described in Table 1; (2) historical reports of changes in lake level during the nineteenth and early twentieth century; (3) Apewu gauge data (1934 to present). Horizontal bars represent error on dates (standard error for ^{14}C; estimated range for historical dates). R, dates from ref. 9; J, date from ref. 8. Note log time scale.

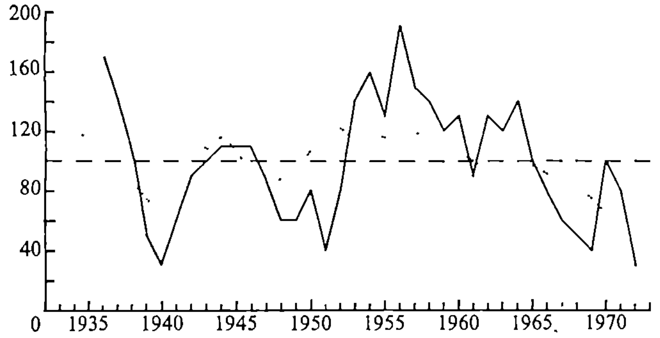


Fig. 3 Five-year running means of monsoon rainfall in the Sahel zone (from ref. 16, Fig. 2) and increments in the level of Lake Bosumtwi (from Apewu gauge record), both expressed as percentage deviation from the mean. . . . . Sahel; ——Lake Bosumtwi.

falls, there seems no reason to suspect an exceptionally different rainfall regime at that time, either. The abrupt decline of Lake Bosumtwi is unlikely, therefore, to have been the result of drastically lower rainfall, but was more probably due to the combined effects of a rise in temperature and accompanying slight decrease in precipitation inducing evaporation losses to exceed water input. An inverse relationship has been demonstrated to exist between temperature and precipitation in some parts of tropical Africa[4,16]. In the Bosumtwi region, during the 1967–1974 dry period, annual precipitation was 92% of the 1932–1974 mean, but temperatures were around 0.2 °C higher than the long-term average. If 1967–1974 conditions were to become the norm, a further rise in mean annual temperature of only 0.5 °C might be sufficient to cause the transition to a negative hydrological balance[10]. As such a rise would probably be accompanied by an even greater decrease in rainfall, it is obvious that only a slight deviation from climatic conditions that have prevailed at times during the twentieth century would be necessary to initiate a regression of Lake Bosumtwi.

Evidence of elevated tropical temperatures in the twelfth and thirteenth centuries should not be altogether unexpected. This was a period of maximum solar activity[17], and there are abundant indications of notable warmth in the temperate northern hemisphere during late Mediaeval times[18–20]. Records of much reduced discharge of the Nile, which must in part have been due to lowering of the great lakes of East Africa[21,22], are perhaps further testimony to accelerated evaporation in the equatorial zone at that time, while climatically-induced environmental stress during the thirteenth and fourteenth centuries may also have been responsible for the rather sudden migration of pastoralists from the Bahr-el-Ghazal region of the Sudan southwards into Uganda[23]. Lamb[16] has observed that the conjunction of lowered equatorial rainfall with periods of increased temperature, and thus greater moisture input into the atmosphere, must imply significant transport of moisture out of the equatorial zone. Such a process could have been particularly intense during the late Mediaeval thermal optimum, when poleward displacement of moist tropical air may have helped bring about the widely reported exceptional precipitation that affected higher latitudes at that time[18–20].

Identifying the cause of the mid-Holocene fall of Lake Bosumtwi must await more precise dating of this event. It was probably coeval with the general decline of African lakes, Saharan, Sahelian and equatorial, which commenced between 6,000 yr BP and 5,000 yr BP[1–3]. Diverse sorts of evidence indicate that the period 6,000yr BP–4,000 yr BP was everywhere characterised by relative warmth[20,24,29], so elevated evaporation rates are likely to have played an important part in the fall of Lake Bosumtwi at that time as well.

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Final results of study of infants at risk of sudden death

IN 1974 we described how we had used routine obstetric and perinatal records to construct a scoring system for identifying within 24 h of birth infants at risk of dying unexpectedly, and we reported the results of the first year of a prospective evaluation of the system[1]. We now have the final results of the study which confirm that the scoring system is effective. The study data and subsequent work suggest that deaths can be reduced by increased surveillance of high risk infants.

The scoring system used combined observations of eight variables[2]—mother's age and blood group, whether she was reported as having a urinary infection or polyhydramnios during pregnancy, the duration of the second stage of labour, the birth order of the infant, whether it was premature, and whether the mother intended to breast feed. Increasing age of mother, blood group A and breast feeding were associated with low risk. The high risk infants were not identified by single factors as is the common practice[3–5] but by the weighted combination of all observations.

The organisation of the prospective study[6] was briefly as follows. Each week day the score of each new baby was calculated. Babies born at home were included. Infants scoring above the estimated 85th quantile were designated high risk. After excluding high risk infants with gross congenital anomalies, the high risk infants were randomly allocated to 'observation' or control groups. At certain times because of holidays, all high risk infants were allocated to the control group. The observation group were invited to participate in the study. This comprised a clinical examination made within 48 h of birth, a second at 5 weeks, together with 10 visits to the home spread over the first 20 weeks of life by specially appointed health visitors. Half of the infants who dropped out of the follow-up study are included in the study group and half with the refused. All babies born in 1973 and 1974 in the County Borough of Sheffield, as defined before the local government reorganisation, were included in the prospective study. By the end of 1976 all these infants were at least 2 yr old, the generally accepted upper age limit for the occurrence of unexpected infant death[7].

The results are summarised in Table 1. A total of 11,424 infants were scored. Our count for 1973 differs by 6 from that published by the Office of Population Censuses and Surveys. Due to local government reorganisation no official figures are available for the study area for 1974, but our study continued unaltered and we think few if any babies were not scored. In all 15.7% of infants were scored as high risk.

Table 1 Post perinatal infant mortality, that is, 1 week—1 year, in various groups of the prospective study.

	No. in group	Probably unpreventable deaths	Unexpected deaths	
			No.	‰ of group
High risk				
Observed	627	1	2	3.2
Control	922	4	9	9.8
Refused	210	—	3	14.3
Excluded	35	12	1	—
Low risk	9,630	12	15	1.6
Total	11,424	29	30	2.6

Deaths are shown under two headings, probably unpreventable and unexpected deaths[8]. Post-mortem assessments were made without knowledge of the high risk grouping. Deaths due to anomalies, for example congenital heart defect, tumour or Werdig-Hoffman syndrome together with deaths of infants who never left hospital are shown as probably unpreventable. One high risk infant excluded and kept in hospital several weeks because of a suspected congenital heart defect, died unexpectedly a week after discharge, but the anomaly could not be demonstrated post mortem. Table 1 shows that the unexpected death rate in the high risk control group is more than 6 times that of the low risk group (relative risk 6.3). This difference is unlikely to occur by chance ($P < 0.001$), and shows that the scoring system is highly effective. The relative risk of unexpected death in the group that refused is 9.2 and is also statistically significantly greater than 1, $P < 0.001$. The relative risk of the observed group is only twice that of the low risk group.

Between the age of 20 weeks and 1 yr there were three unexpected deaths, one in the high risk control group and two in the low risk group. In addition, there were five unexpected deaths between the ages of 1 and 2 yr, one in each of the first three high risk groups and two in the low risk group. The results are therefore essentially the same if the data are examined at 20 weeks, at age 1 yr as in Table 1, or at 2 yr. Overall these final results are remarkably similar to those previously reported[1].

The possibility that surveillance reduces mortality requires comment. The difference between the unexpected death rate in the studied group and the high risk control group is not statistically significant using a χ^2 test. If we group those who refused surveillance with the controls, the chance of a difference as large or larger, using an exact one-sided test of significance, is 0.076.

Our primary objective was to observe the high risk group, but to ignore serious medical conditions would have been unethical. By special arrangement our team of visitors were allowed to take infants directly to hospital if necessary and when the 5 week clinical examination showed that bottle feeds were often over-concentrated[9] active steps were taken to ensure that feeds were correctly made up. It is possible to dismiss the reduction in the unexpected death rate in the study group as non-significant, as Magura has done[10], but the alternative hypothesis that the death rate is reduced is more likely.

The difference between study and control groups is also less different than might be expected because all groups benefited from the study, for example, the campaign to explain the dangers of overstrength feeding was not confined to the study group. The benefit is reflected in a reduction in the post perinatal mortality rate. For 1968–1972 the average post perinatal infant mortality rate for Sheffield was 8.2 per thousand, compared with a rate of 7.6 per thousand in England and Wales in this period. For the study period the rate was 5.2 per thousand—England and Wales 7.4. Had the observed group had the same mortality rate as the control group, the rate would have been 5.7 per thousand. On the other hand, if the benefits of observation were extended to all high risk infants a mortality rate of 4.8 per thousand might be expected.

Funds did not permit the prolongation of the study to enable us to establish unequivocally the benefits of surveillance or to determine which components reduce mortality. But, since January 1975 all infants born in the new County District of Sheffield have been scored and the community health authorities, including the health visitors, have been regularly notified of all high risk infants. In addition since August 1975 a new system has been introduced whereby all infants are examined in the home by a health visitor at the age of 4 weeks. The post perinatal infant mortality rate for 1975 and 1976 is 4.7 per thousand, which is almost exactly the rate expected if surveillance is effective, and is statistically significantly lower than 6.3 per thousand, the post perinatal mortality rate for these years in England and Wales, $P < 0.01$.

We conclude that infants at increased risk of unexpected post perinatal death can be identified at birth. Our data also suggest that post perinatal deaths may be prevented by alerting the primary health care nurses to the surveillance of this group. We have also recently shown that the identification of high risk children can be made more effective by encorporating information on the early development of the child by a multi-stage scoring system. In a small prospective test 71% of unexpected deaths were allocated to the high risk group which had a relative risk of 9.2 (ref. 11). We are currently planning to implement multi-stage scoring.

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Hypolimnas bolina (L.), a mimic of danaid butterflies, and its model *Euploea core* (Cram.) store cardioactive substances

Hypolimnas bolina is a sexually dimorphic nymphalid butterfly with a west to east distribution from Madagascar to Easter Island, and north to south from Japan to Australasia. To the west the female is monomorphic, mimicking species of the oriental and Australasian danaid genus *Euploea*[1]. Eastwards *H. bolina* is frequently polymorphic and most forms are then non-mimetic. In areas where it resembles *Euploea* the butterfly has usually been designated a Batesian mimic. The supposed model feeds on Asclepiadaceae and Moraceae only, two families of plants of which various species possess emetic, toxic and purgative properties, whereas the foodplants of *Hypolimnas* include examples from eleven families, only a few of which are irritant or toxic[2]. We now report that when the larva of *H bolina* feeds on *Ipomoea batatas*, the butterfly stores cardio-active substances. Dried specimens of this butterfly, reared on *Asystasia gangetica*, a plant which does not contain these substances (Table 1), evoke no reaction on isolated rat heart, but living specimens produce a slight response. This indicates that in addition to material sequestered from foodplants, the butterfly itself secretes some type of cardioactive substance. A loss of intrinsic toxic qualities after death has been demonstrated in almost all aposematic model Lepidoptera[3], whereas stored secondary plant substances can usually be recorded from an analysis of dried material.

Possibly relevant to our findings with *H. bolina* is the fact that this butterfly has several features unusual in classical Batesian mimics: (1) the mimetic pattern has not broken down in Madagascar where the model is lacking; (2) although polymorphic—like many Batesian mimics—several of the forms are non-*Euploea*-like and have no recognised model butterflies flying with them; (3) *H. bolina* is remarkably long-lived, a characteristic usually associated with toxic models rather than innocuous mimics; (4) inheritance of the wing pattern is different from that found in the mimetic papilios[1]. In *H. bolina* there is no genetic linkage between the locus controlling the mimicry and that responsible for the other polymorphic forms[1].

We have investigated the toxicity or pharmacological activity of *H. bolina* and its model *Euploea core*, extracts of the butterflies and their foodplants being tested on the isolated rat and chicken heart (Langendorff preparation[4]) and fed by gavage to pigeons. The insects were bred as described before[1] and their origins are shown in Table 1.

Our results are set out in Tables 1 and 2 and Fig. 1. As Table 1 shows, chicken heart responded to extracts of the model *E. core*, reared either on *Nerium oleander* or three *Asclepias* species, by a reduction in both heart rate and strength of contraction. This suggests that *E. core* stores cardenolides, presumably sequestered from the foodplant[5], although other cardioactive substances may also be present, masked by plant material. But no emetic effects were produced by feeding pigeons with extracts of either fresh or dried adults or pupae (several trials), showing that the butterfly is not such an effective storer of cardiac glycosides as another model, *Danaus plexippus* (ref. 6 and C. A. Dixon, J. M. Erickson, M.R. and D.N.K., in preparation). The situation is reminiscent of that recorded for the African model of the related *Hypolimnas misippus*, *Danaus chrysippus*[7,8], in which sequestration and storage is less effective

Table 1 Effects of adult butterfly extracts on isolated heart

	Foodplant	Sex	No. of experiments	Av. no. of insects extracted per experiment	Av. wt of insects extracted per dose (mg)	Heart tested	% Change in heart rate (mean and range)	% Change in strength of contraction (mean and range)
Euploea core asela Sri Lanka	*Nerium oleander*	Female	6	3.1	86	Chicken	−32.8 (−20, −60)	−26.5 (−11, −66)
		Male	4	2.8	101	Chicken	−16.8 (0, −65)	−13.8 (0, −48)
	Asclepias curassavica	Female	1	3	59	Chicken	−19	−11
	Gomphocarpus fruticosus	Male	1	3	84	Rat	−28	+20
Euploea core amymone Hong Kong	*Nerium oleander*	Female	1	4	73	Chicken	−26	−26
		Male	1	3	58	Rat	−37	+32
	Asclepias curassavica	Male	1	3	191	Rat*	−29	+23
	Gomphocarpus fruticosus	Female	1	2	60	Chicken	−15	−15
		Female	2	3	126	Rat†	−30 (−30)	+21 (+16, +26)
		Male	1	3	191	Rat	−29	+23
	Calotropis procera	Female	1	2	59	Chicken	−21	−16
		Male	2	3	139	Rat	−24.5 (−19, −30)	+18 (+16, +20)
Hypolimnas bolina Sri Lanka, Fiji, Sabah	*Ipomoea batatas*	Female	3	5	89	Rat‡	−42 (−5, −71)	+51 (+20, +66)
	Asystasia gangetica	Female (dried material)	1	5	33	Rat	0	0
		Male (dried material)	1	7	44	Rat	0	0
		Female (fresh material)	1	5	121	Rat	Trace of cardiac activity	—
		Male (fresh material)	1	5	58	Rat	Trace of cardiac activity	—

Euploea extracts were made from live butterflies, *Hypolimnas* extracts from dried material (storage time 2–3 yr) and live material. Extracts from *Hypolimnas* females fed on *Ipomoea*, when injected into the haemocoel of locusts, caused paralysis after 10 min followed by death after 24 h. Neither extracts of dried *Hypolimnas* nor extracts of fresh or dried *Euploea* caused emesis after administration to the pigeon by gavage. Heart rate and strength of contraction were measured for 30 s beginning 10 s after the injection of extract. The control injection effect due to the saline diluent used in mixing extracts is seen in all three figures as a drop in strength almost immediately after injection. Figure 1*a* shows the less common injection artefact of raising the baseline.

* See Fig. 1*b*.

† See Fig. 1*a*.

‡ See Fig. 1*c*.

and more variable, depending not only on the type of foodplant but on other, probably genetic, factors.

There seemed to be no consistent differences between the cardioactivity produced by the two subspecies of the model *E. core asela* and *E. core amymone*, or by either sex, when fed on the same plants.

Hypolimnas bolina (Table 1), when reared on *Ipomoea batatas* (Convolvulaceae), contained cardioactive substances which also reduced heart rate and increased strength of contraction of the rat heart. Similar activity was produced by extracts of the foodplant *Ipomoea* (Table 2), which genus contains an unusually high proportion of aposematic herbivores[9].

Although the concept of mimicry is now firmly established there is still discussion about the limitations of Batesian and Müllerian categories. It has recently been recognised that the role of model and mimic can vary according to local conditions and geographical distribution[7,8,10]. Thus a model can be more or less toxic or distasteful according to the secondary plant substances available in its diet.

Our experiments with captive and wild birds (ref. 1 and unpublished results of M.R.) have shown that *H. bolina* is not a distasteful species for Corvidae, passerines and quails (*Coturnix*), but that *E. core*, in spite of its lack of emetic qualities, is rejected by these captive birds (unpublished results of M.R.). The presence of cardioactive substances in *H. bolina* when it is reared on *I. batatas* indicates that this butterfly is also an opportunistic sequesterer of active secondary plant substances, and can probably acquire other deterrents such as pyrrolizidine alkaloids from *Senecio* (a recorded foodplant) should the opportunity arise. The foliage of *I. batatas* has been suspected of poisoning stock in Arizona and Australia[11,12] and there is a report of a severe outbreak of gastro-enteritis in a Bombay jail traced to this source[13].

In addition to specific glycoresins with purgative activity, *Ipomoea* has been shown to contain indole derivatives (containing the nucleus of lysergic acid) and dendrolasin[14], a sesquiterpine. Any of these toxic substances might be sequestered by *H. bolina* from a particular strain of *I. batatas*.

Hypolimnas is a genus which contains about 30 species, two-thirds of which are mimics of danaids. From our

Fig. 1 On each figure the horizontal line indicates 10 s; extracts were given at the points shown by the arrows. Fluctuations in heart rate and strength of contraction in the first 6–10 s after administration of the extract are injection artefacts and have been ignored in the calculations of results shown in Table 1. *a*, Effect of *Euploea core amymone* (*Gomphocarpus fruticosus*-fed females) extract on the rate and strength of contraction of the isolated rat heart (each vertical trace represents one heart beat). *b*, Effect of *Euploea core amymone* (*A. curassavica*-fed males) extract on the isolated rat heart. *c*, Effect of *Hypolimnas bolina* (*Ipomoea*-fed females) extract on the isolated rat heart.

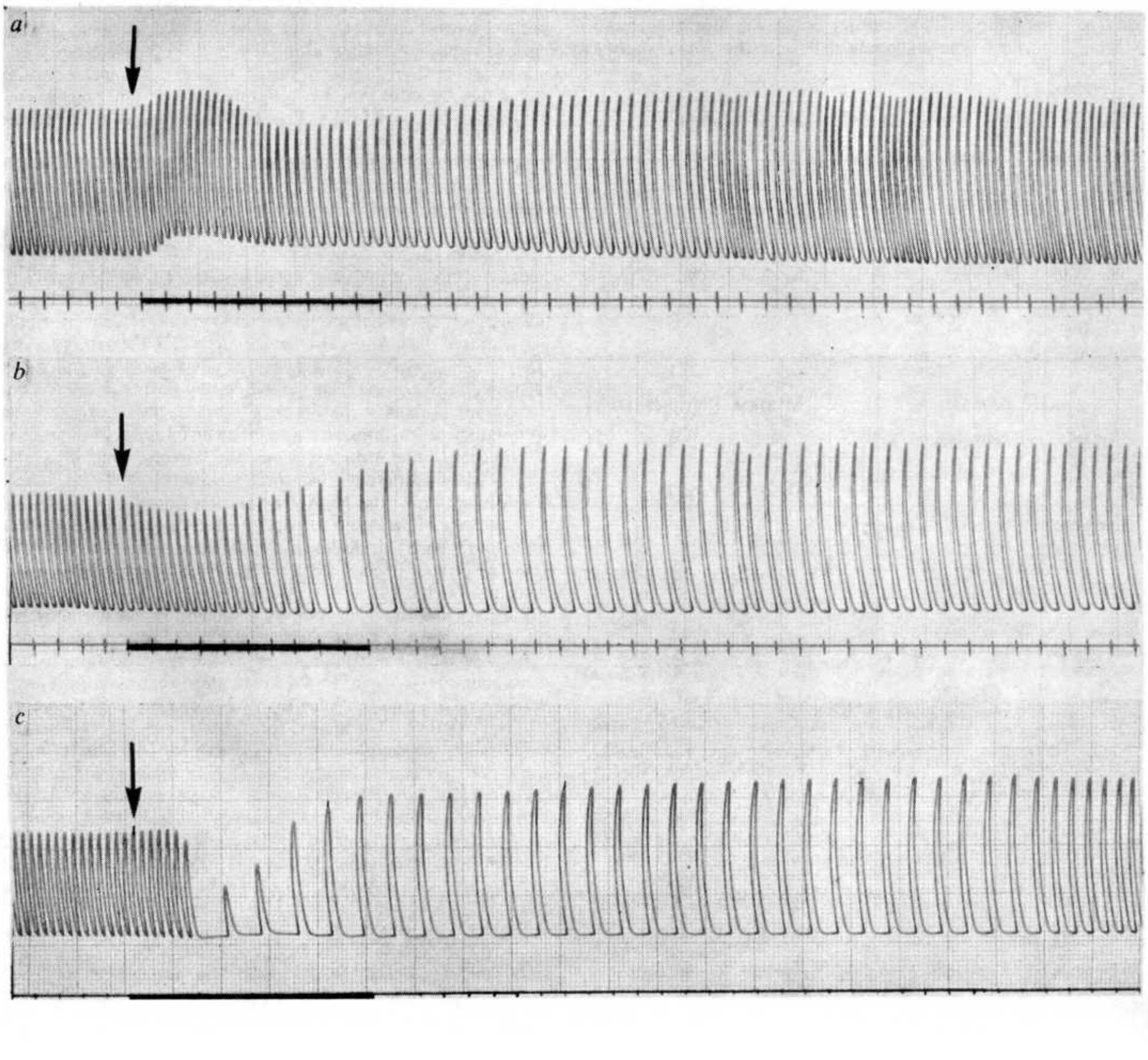

Table 2 Effects of foodplant extracts on the isolated heart and on the pigeon

	No. of experiments	Av. wt of plant extracted per dose (mg)	Heart tested	% Change in heart rate (mean and range)	% Change strength of contraction (mean and range)	Effect of feeding extract of foodplant by gavage*
Ipomoea	3	111	Rat	−47 (−3, −100)	+36 (0, +86)	0
batatas	1	58	Chicken	−15	−8	
Asystasia	1	60	Rat	0	0	ND
gangetica	1	60	Chicken	0	0	
Nerium	1	114	Rat	−16	−41	+++
oleander	1	157	Chicken	+100	+72	
Asclepias	2	115	Rat	−58.5 (−52, −65)	−10 (−5, −15)	+
curassavica	1	160	Chicken	−33	+36	
Gomphocarpus				ND	ND	+++
fruticosus						
Calotropis				ND	ND	++
procera						

Extracts were of foliage, except for *Ipomoea* for which both stems and foliage were extracted. Extracts of *Ipomoea* had no effect on the locust after injection into the haemocoel.

*ND, not done; +++, vomiting within 5 min; ++, within 6–14 min; +, within 15–30 min.

preliminary experiments we draw the tentative conclusion that *H. bolina* has a special type of Müllerian mimicry relationship with *Euploea*, and according to circumstances and the species of foodplant available, either species can play the part of model or mimic and have co-equal 'common warning coloration'. Others[15–17] have drawn attention to the unusual distribution of *H. misippus* and its model, and it seems likely that this species, which at times also feeds on *Ipomoea* (unpublished results of David Smith) demonstrates a similar type of relationship to that of *H. bolina* and *E. core*.

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Decay in juvenile hormone biosynthesis by insect corpus allatum after nerve transection

THE ability of the insect corpus allatum (CA) to synthesise and release juvenile hormone (JH) is believed to be controlled by various neural and humoral influences of both stimulatory and inhibitory nature[1]. In last instar *Galleria mellonella*, a CA stimulatory neurohormone (allatotropin) originating in the medial neurosecretory cells of the brain and operating during the early part of the instar has been proposed[2,4]. In the later part of the instar the synthesis and/or release of JH is inhibited by nervous impulses originating in the brain and reaching the CA through nervous connections[2]. A similar nervous inhibition of JH synthesis is thought to operate in adult *Pyrrhocoris apterus*[5] and in the cockroaches *Leucophaea maderae*[6] and *Diploptera punctata*[7], because section of the nervi corporis allati 1 (NCA 1) seems to activate the CA. A neurohormonal factor from the medial neurosecretory cells of the pars intercerebralis which causes the activation of the CA has also been proposed in the orthopterans, *Anacridium aegyptium* and *Locusta migratoria*[8]. In still other Orthoptera, *Schistocerca* sp., the necessity for intact NCA 1 for activation of the CA has been suggested, indicating a direct stimulatory neural influence rather than an inhibitory influence or a humoral stimulation[9,10]. Thus the factors controlling JH biosynthesis in different insect species are not clear. We show here that disruption of the NCA 1 by section during the first gonotrophic cycle of the desert locust *Schistocerca gregaria* results in a rapid and predictable decline in the ability of the CA to synthesise JH. The effect of section of the NCA 1 on JH biosynthesis is detectable within 3 h.

To determine the effect of nerve section on JH biosynthesis, the NCA 1 to each CA was severed at selected times during the first gonotrophic cycle of female locusts. After nerve section, locusts were maintained for up to 4 d in standard rearing conditions[11] before assaying the rate of biosynthesis of JH (C_{16}JH—methyl 10,11-epoxy-3, 7, 11-trimethyl-*trans, trans*-2, 6-dodecadienoate) using our *in vitro* radiochemical procedures[12,13]. Observations were made on glands from locusts during the first gonotrophic cycle (8–13 d after fledging) and changes in the rate of JH biosynthesis of up to several hundredfold have been reported previously during this period[11]. Maximal rates of JH biosynthesis have been observed to occur on day 11 (ref. 11) and the effect of nerve section on biosynthetic rates may be most easily observed at this time.

Figure 1 shows the rates of synthesis of JH during the first gonotrophic cycle for operated locusts (NCA 1 severed) and for sham-operated locusts (NCA 1 touched but not severed) between 1 and 4 d after the operation. The lines join the median values for each day. Differences between median values for the various times after nerve section are small on any given day (on days 9 and 11), and cannot be distinguished in Fig 1. At all times the median JH biosynthetic rate in sham-operated animals was greater than that in operated animals The median values for operated animals were consistently low throughout the experiment and no differences were apparent between animals in which the NCA 1 were severed for various times. The sham-operated values reached a maximum on day 11, in good agreement with results for unoperated normal animals[11]. These results indicate that within 1 d of section of the NCA 1, the CA lost the ability to biosynthesise JH, and did not regain it for at least 4 d.

Because of this rapid loss in CA activity, the JH biosynthetic abilities of CA were determined at 3–6 h intervals during the first 24 h after the operation Locusts 10–11 d after fledging were used because of the high JH biosynthetic rates observed at this time. Figure 2 shows that CA activity again decreased rapidly after nerve section; within 3 h values for operated animals were consistently lower than those for sham-operated animals, and by 6 h, JH biosynthesis by CA of operated animals was low The assay procedure included a 3 h incubation of CA *in vitro* so that the values in Fig. 2 represent the rates of JH biosynthesis during a 3 h interval after the elapsed post-operative period. Thus the values at 3 h, for example, actually represent the rate of JH biosynthesis between 3 and 6 h after the initial operation; similarly, 12 h values represent the rate of biosynthesis between 12 and 15 h after nerve section.

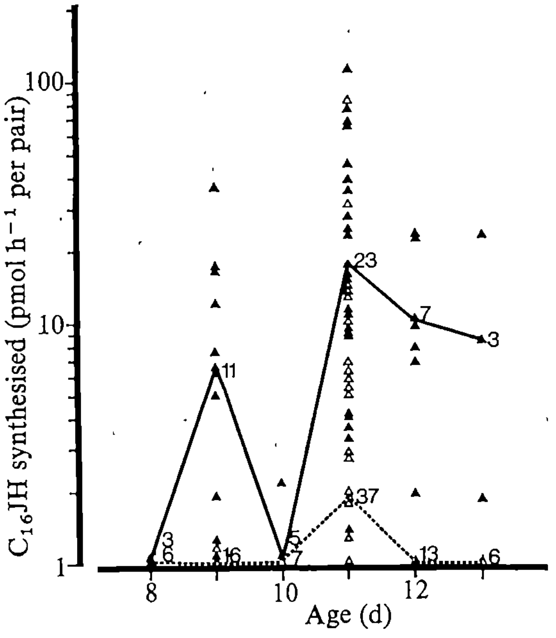

Fig. 1 The rate of synthesis of C_{16}JH by corpora allata during the first gonotrophic cycle of *Schistocerca gregaria* at various times after section of the NCA 1. Values on day 8, 2 d after nerve section, day 9—2 and 3 d after section, day 10, 3 d after section, day 11, 1 and 4 d after section, day 12, 2 d after section, day 13, 3 d after section Each point represents a single determination on an individual pair of glands Δ, Animals in which the NCA 1 was sectioned, ▲, sham-operated animals The dotted line joins the median values of NCA 1-sectioned animals and the solid line joins the median values of sham-operated animals The numbers adjacent to the median values represent the total number of determinations on any given day In every case, the median rates of synthesis in operated animals are lower than the sham-operated animals and never greater than 2 pmol per h per pair The rates of synthesis of C_{16}JH were monitored by incubation of the glands for 3 h in TC 199 (GIBCO, 25 mM HEPES) containing Ficoll (20 mg . ml^{-1}) and methyl ^{14}C-methionine (Amersham–Searle, final specific radioactivity 35–37 mCi mmol^{-1}) After incubation, glands plus medium were extracted with 15 volumes of $CHCl_3$ C_2H_5OH (5·1) after adjustment to *p*H 4 6 and addition of appropriate non-radioactive marker compounds Extracts were analysed by thin layer chromatography (Merck F_{254} silica gel) in ethyl acetate *n*-hexane (25 75) Radioactivity in C_{16}JH was quantified by liquid scintillation spectrometry.

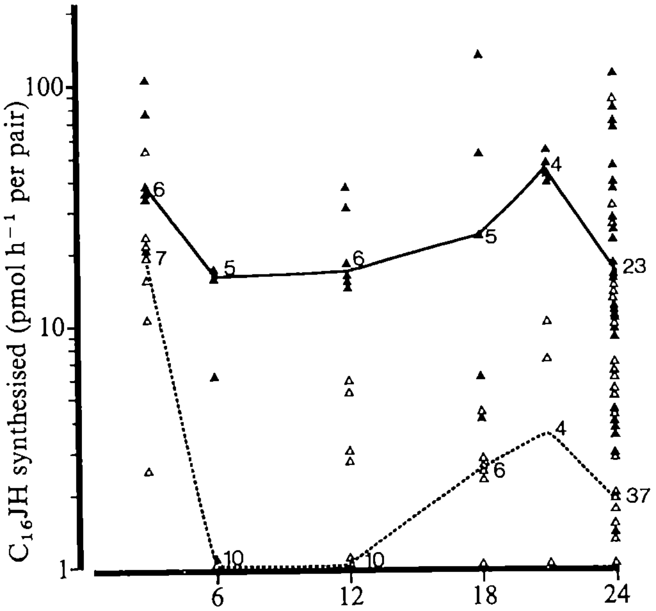

Fig. 2 The rate of synthesis of C_{16}JH by corpora allata on day 11 after fledging at various times after section of the NCA 1 Each point represents a single determination on an individual pair of glands Symbols and methods as for Fig 1 In every case, the median rates of synthesis in operated animals are lower than those of the sham-operated animals The effect of nerve section can be seen within 3 h of the operation and by 6 h, the rate of synthesis has dropped to a low level Section of the NCA 1 results in a rapid decline in C_{16}JH biosynthesis

It is useful to compare the decay in CA activity after nerve section with the time course of release of JH by locust CA *in vitro* (completely denervated) It has been shown that JH is released at a constant rate from isolated CA of *S gregaria* for 3 h *in vitro*[14] and release then declines rapidly, principally in CA with high synthetic activity. Our results *in vivo* confirm these observations because there was a significant decrease in the rate of JH biosynthesis within 6 h of nerve section, that is, 3 h after nerve section *in vivo* + 3 h incubation *in vitro*. CA with low rates of JH release (low activity CA), however, continue to release JH at a constant rate for at least 5 h (ref. 14). These CA *in vitro* have rates of biosynthesis similar to both those CA with high synthetic activity after 3 h *in vitro* and those CA observed *in vitro* after nerve section. This suggests that CA lacking the appropriate 'neural' input synthesise JH at a low but constant rate for some time, whether *in vivo* or *in vitro* Section of the NCA 1 in animals with active CA therefore resulted in a rapid decline in CA activity whereas section of the NCA 1 in animals with CA of low synthetic activity did not significantly alter the rate of JH biosynthesis. There was a large between-animal variation in the rates of biosynthesis in both unoperated locusts[11] and in sham-operated animals (Figs 1 and 2) and we attribute much of this to the difficulty in obtaining completely synchronised populations of animals, when the activity of the CA is changing so rapidly[11]. Because not all of the experimental CA exhibit high activity at any given time, the effect of nerve section on CA activity is probably more pronounced than Figs 1 and 2 indicate.

It may be suggested that section of the NCA 1 damages the CA, thus altering their ability to synthesise JH. To test this possibility, we incubated CA from both operated and sham-operated animals at two times after nerve section, in the presence of the exogenous precursor farnesenic acid. Farnesenic acid has been demonstrated previously to undergo rapid conversion to C_{16}JH through esterification and epoxidation[12,15], and any damage to the cells of the CA presumably would be reflected in an impaired ability to effect these terminal critical steps in JH biosynthesis. Table 1 shows the biosynthetic rates by CA from 11-d locusts 12 and 24 h after section of the NCA 1, incubated in the presence of farnesenic acid. *t* tests on these values at each time revealed that there was no

Table 1 $C_{16}JH$ synthesis *in vitro* in presence of farnesenic acid after section of the NCA 1

Adult age (d)	Time after section of NCA 1 (h)	$C_{16}JH$ Synthesis (pmol per h per pair) Operated	Sham-operated
11	12	Mean 167.9 ± 20.4 Median 171.5 ($n = 10$)	181.1 ± 23.2 183.1 ($n = 8$)
11	24	Mean 95.1 ± 7.62 Median 95.1 ($n = 7$)	121.3 ± 13.0 118.0 ($n = 7$)

Mean values are ± s.e.m; *n*, no. of animals assayed. There is no significant difference between operated and sham-operated values. At 12 h, $P > 0.6$ and at 24 h, $P > 0.05$.

significant difference in JH biosynthesis between operated and sham-operated CA ($P > 0.6$ at 12 h; $P > 0.05$ at 24 h). This demonstrates that section of the NCA 1 does not alter the ability of the CA to effect the final two stages in JH biosynthesis. In addition, these results suggest that the stimulatory or sustaining factor(s) arriving at the CA through the NCA 1 do not exert their effects by altering the activities of the two terminal enzymic stages. It has been suggested that rate limitation in JH biosynthesis does not occur at the final two stages in *S. gregaria*[15], and our results support this suggestion.

Our results show that the presence of intact NCA 1 is necessary for the continued biosynthesis of JH by active CA in this species and provide a possible explanation for the difficulties encountered by some authors when attempting to promote sexual maturation or the expression of juvenile characters through the implantation of homologous CA (refs 16–18). The CA from adults of certain other species seem to respond differently. For example, CA from female *Periplaneta americana* take more than 36 h to reduce their rate of incorporation of ^{14}C-methionine into $C_{16}JH$ when isolated in chemically-defined culture medium[20], and there is physiological evidence that these glands may retain significant levels of synthetic activity for several weeks when transplanted to larval *Rhodnius prolixus*[21]. These other CA may be less acutely dependent upon the integrity of their nervous connection to the brain than those of *S. gregaria*, and in some cases may be able to respond independently to trophic humoral factors if these are present.

Our results suggest that if a stimulatory or sustaining factor regulates JH biosynthesis in adult *S. gregaria*, this factor does not normally operate by a humoral pathway. Massive electrical stimulation of the pars intercerebralis of *L. migratoria* and *A. aegyptium* has been reported to result in activation of denervated inactive CA (ref. 8). We suggest that although massive release of neurosecretory material may result in activation of the CA by a humoral pathway in experimental conditions, this is not the normal pathway in *Schistocerca*. Rather the factor must reach the CA by the nervous tracts. Other authors have shown that electrical stimulation of the brain in *S. gregaria* also results in activation of *intact* CA (ref. 19). Thus activation may occur by conventional nervous pathways as well. It is not known if the 'allatotropic' factor is the product of neurosecretory cells or conventional neurones or if both types of products are involved in the activation of the CA. With regard to the latter possibility, conventional neurones may regulate the release of neurosecretory products within the CA. Such a mechanism may regulate the release of egg-laying hormone (a neurosecretion) in *Aplysia*[22,23].

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Sex hormone of *Dictyostelium discoideum* is volatile

THE macrocyst pathway of development represents the sexual cycle of the cellular slime moulds[1–4]. O'Day and Lewis[5] have shown that the sexual interaction between opposite mating types of *Dictyostelium discoideum* is mediated by a low molecular weight, diffusible hormone. They also showed that only one mating-type strain (NC-4) was a secretor of the sexual hormone while the opposite strain (V-12) was a responder (that is, formed macrocysts). This sexual interaction was verified by MacHac and Bonner[6] and was later shown to exist in another species, *D. purpureum*[7]. In our attempts to characterise the sexual system of *D. discoideum*, our results have revealed that the sexual hormone of this species is volatile.

Figure 1 depicts the construction of the diffusion chamber and outlines the culture methods used in this study. The stock cultures of the mating-type strains (NC-4 and V-12)

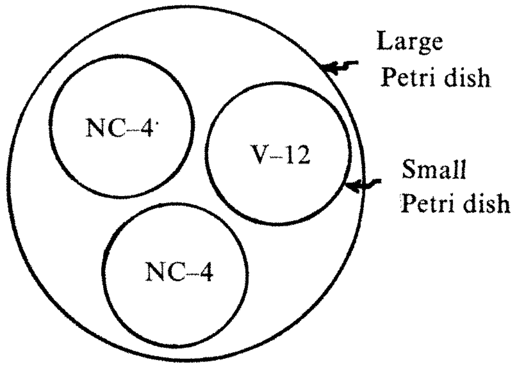

Fig. 1 Diagram of the diffusion chamber with an example of the most successful inducing situation. Small Falcon plastic Petri dishes (30 × 10 mm) containing 4.0 ml 0.1% L-P agar (0.1% Difco peptone, 0.1% Difco lactose, 1.5% Difco agar) were inoculated with 0.5–1.0 ml suspension of *E. coli* B/r and spores (1–2.5 × 10^4 spores) of one mating-type strain of *D. discoideum*, Three of these small dishes were placed uncovered in a large Fisher plastic Petri dish (100 × 15 mm) which was then covered and sealed with tape (masking tape or 3M vinyl electrical tape). The sealed plates were placed in light-tight metal canisters and stored for two weeks at 23 ± 1 °C before observation.

Table 1 Macrocyst formation in diffusion chamber cultures

Experimental situation	No. of chambers set up	No. of small plates with pre-cyst clumps*		No. of small plates with mature macrocysts*	
		V-12	NC-4	V-12	NC-4
Two NC-4; one V-12	42	30/42	0/84	6/42	0/84
Two V-12; one NC-4	20	5/40	0/20	0/40	0/20
Two d-H_2O; one V-12	10	0/10	NA	0/10	NA
Three NC-4	20	NA	0/60	NA	0/60
Three V-12	20	0/60	NA	0/60	NA

*Macrocyst formation involves an initial aggregation of cells to form discrete, tight cell clumps (pre-cyst clumps) which, in appropriate conditions, form dormant structures possessing three cell walls (mature macrocyst). Data are expressed as ratios of the total number of small plates with pre-cyst clumps or mature macrocysts to total number of small plates used for that strain.

used to inoculate the small Petri dishes were maintained on *Escherichia coli* strain B/r on 0.1% L-P agar at 22–24 °C in the presence of light[8]. The three small plates of the diffusion chamber were inoculated in the following combinations: two NC-4 and one V-12; two V-12 and one NC-4; two distilled water and one V-12; three NC-4; three V-12. After two weeks the small plates of each chamber were examined for the presence of macrocysts.

The results of these experiments are summarised in Table 1. Pre-cyst cell clumps and mature macrocysts were only observed in cultures of V-12. Furthermore, these structures were only observed when at least one NC-4 culture was present in the diffusion chamber, whereas mature macrocysts were only observed when two NC-4 cultures were present. In other experiments, seven of the small Petri dishes (six NC-4; one V-12) were placed in a very large, sealed, plastic Petri dish (150×20 mm) in an attempt to increase the level of macrocyst formation. Only a low level of macrocyst formation by V-12, however, was observed, possibly due to the large air space in such containers. The fact that only V-12 cultures contained macrocysts argues that cell migration from one small dish to another did not occur. As an additional control, however, in several experiments a 'moat' of sterile, distilled water was placed around the base of the small Petri dish containing the V-12 culture. After the two-week period, the contents of the moat were examined by phase contrast microscopy. Amoebae were never observed in this fluid, re-emphasising that cellular migration between plates did not occur.

In a different but related series of experiments, ten mixed mating-type cultures of NC-4 and V-12 were left unsealed, whereas ten were sealed. In these experiments, the sealed dishes routinely showed 500 or more macrocysts per plate, whereas the unsealed cultures had 50–100 or fewer.

The results thus reveal that the sexual hormone of *D. discoideum* is volatile. This organism, however, is not unique, since volatile sex hormones are produced by many other eukaryotic microbes[9]. In addition, volatile molecules may play a role in other aspects of slime mould development such as in the spacing of fruiting bodies, in the formation of microcysts and in homothallism[10,11]. The data are also consistent with the concept that macrocyst formation is at least a two-step phenomenon. Since pre-cyst clumps only formed in cultures which had a ratio of one NC-4 to two V-12, whereas mature macrocysts were formed by V-12 when two NC-4 cultures were present, it is likely that a low level of hormone may induce pre-cyst formation, while a higher level of this hormone or, possibly, a second volatile molecule may be required for events to proceed past this point.

Finally, the fact that the aggregation-inducing factor for sexual interaction is volatile at 23±1 °C, whereas cyclic AMP is not, reveals that the events of cell aggregation in these two alternative developmental events are different. Presently we are continuing our attempts to identify the sexual hormone of *D. discoideum*.

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Modification of circadian flight activity in the mosquito *Anopheles gambiae* after insemination

THE flight activity of female *Anopheles gambiae* Giles, recorded in the laboratory, follows a bimodal cycle[1]. In LD12:12 (alternating 12 h light:12 h dark) the first peak comes shortly after light-off and there is a secondary phase of activity which reaches its maximum in the middle or latter part of the dark phase. This secondary activity occurs at the time when host seeking and biting take place in the wild[2]; the initial peak corresponds with the time of egress of mosquitoes from their daytime resting places and, in particular, in virgin females, with the time of mating[3]. In normal conditions, *A. gambiae* females are inseminated only once in their lifetime[4,5], and usually not before they are 2–3 d old (ref. 3). We show here that, after insemination, the first peak of activity is greatly reduced and the secondary phase of activity is enhanced. This switch in activity is likely to represent a change from mating to host seeking behaviour.

Pala strain female mosquitoes, were sugar-fed and reared at 26 °C in an LD12:12 regime. Males and females were separated following emergence. When 3–4 d old, half the females were placed in a cage for 24 h with twice the number of males; the insemination rate was approximately 90%. The flight activity of similar numbers of individual virgin and mated females was then recorded for a week, using the acoustic actograph technique[6,7]. The experiments were first carried out in an LD12:12 regime with a sharp change from light to dark and vice versa, and then repeated using an electronically produced artificial dawn and dusk[3] with the transition lasting approximately 75 min. After each experiment, survivors were dissected and spermathecae examined.

Figure 1 shows the mean flight activity of virgin and inseminated females 6 d after emergence, that is, in the case of the

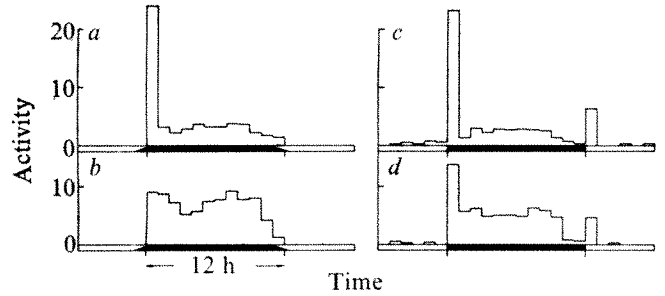

Fig. 1 Flight activity (mean score per h) of sugar-fed *A. gambiae* females in LD12:12. *a*, *b*, With an artificial dawn and dusk: *a*, virgins ($n = 29$) and *b*, inseminated ($n = 28$). *c*, *d*, With sharp light-on and light-off: *c*, virgins ($n = 42$) and *d*, inseminated ($n = 25$).

mated females, 24 h after the end of their confinement with the males. In both types of regime, inseminated females show a significant decrease in amount of activity in the first hour of the dark period (t test, $P < 0.01$ in both cases) and an increase in the secondary activity (t test, $P < 0.02$ in both cases). The net effect is an increase of 30–50% in the total activity during the dark phase. The same pattern was observed throughout the experiment. The difference in activity between virgin and inseminated females is more immediately apparent in dawn and dusk conditions. Possibly the peak shown by the inseminated females in Fig. 1*d* is partly a response to the sharp change at light-off; the peak at light-on has already been shown to be a response to such a sharp change[1].

Craig and his co-workers[8,9] have demonstrated that in *Aedes aegypti* and a number of other mosquitoes, the female is rendered refractory to further insemination through the action of the male accessory gland secretion, which is transferred to the female in the seminal fluid. These secretions may also affect the female by stimulating oviposition, modifying biting behaviour, increasing the rate of digestion of blood meals and possibly enhancing longevity[10,11]. It seems probable that the behavioural changes we have observed are a consequence of the transference of accessory gland substance. The switch in behaviour could be particularly important in a species such as *A. gambiae* in which mating and feeding may take place at entirely different sites.

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2,4-D plasmids and persistence

According to the UN Food and Agricultural Organisation production year book of 1974, 27×10^6 kg of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) were consumed in 1973, making this the most extensively used of all pesticides. One desirable characteristic of 2,4-D, apart from its efficacy as a herbicide, is its lack of persistence in the environment[1], due to degradation by a variety of microorganisms. We have argued that in view of the isolation of plasmids encoding the degradation of naturally occurring aliphatic and aromatic compounds[2], there exists a class of plasmids which specifically encode the degradation of many man-made derivatives of these compounds, in this instance 2,4-D. Using segregation and transfer analyses we have demonstrated that a plasmid or plasmids is involved in 2,4-D degradation. Clearly bacteria have evolved which can degrade many different pesticides; our results indicate that plasmids may have an important role in this evolution.

Initial attempts to study 2,4-D degrading ability in soil bacteria were hampered by the marked instability of this character in many of our isolates. We have isolated a strain of *Pseudomonas* (JMP116) that degrades 2,4-D and retains this characteristic even after subculture in the absence of 2,4-D. Degradation of 2,4-D by JMP116 was detected by acid production in Loos' media[3], disappearance of the 283-nm absorption peak[4] and growth on 2,4-D as a sole source of carbon and energy. Since JMP116 does not degrade phenoxyacetic acid, we have concluded that this pathway is specific for 2,4-D.

In view of the segregation of 2,4-D negative clones by certain of our isolates we examined the possibility that these genes are plasmid borne. We have found that the wild-type JMP116 is readily cured of its 2,4-D degrading ability of mitomycin C. After JMP116 at 10^7 colony-forming units (CFU) per ml was inoculated into PYE broth containing 0.1 μg ml^{-1} mitomycin C and grown at 32 °C with aeration for 18 h, 40–60% of the clones re-isolated on PYE were cured of 2,4-D degrading ability. Control cultures without mitomycin C show < 1% spontaneous loss of this character. Since mitomycin C can act as a mutagen, especially in strains which are sensitive to the antibiotic, we tested one cured isolate (JMP119) for back mutation to the wild type, 2,4-D degrading phenotype. This cured strain does not revert to wild type at a detectable level.

Segregation and curing are characteristics of plasmid genes[5]. For many plasmids it is possible to isolate plasmid-specific bacteriophage[6]. PR11 is a plasmid-specific bacteriophage that formed plaques on strains harbouring RP1, a pseudomonas R plasmid with an exceptionally wide host range[7]. This bacteriophage also plaques on JMP116, but not on JMP119 (a JMP116 derivative cured of its ability to degrade 2,4-D). It is likely that JMP116 harbours a conjugative plasmid related to RP1 that encodes 2,4-D degradation. This has been confirmed by an intrastrain mating between JMP116 and a nalidixic acid resistant mutant of JMP119. The results (Table 1) show that the plasmid is transferred with a frequency of 5×10^{-2} per donor cell in an overnight mating, and there is a 100% correlation between the acquisition of the plasmid and the ability to degrade 2,4-D. Although failure to separate the conjugative phenotype from the 2,4-D degrading phenotype in either curing (unpublished data) or transfer experiments suggests that the 2,4-D degrading ability is encoded by a conjugative plasmid, it must be recognised that more than one plasmid may be involved—for instance a non-conjugative plasmid, specifying the degradative pathway, being mobilised by a conjugative plasmid. Information on the actual number of plasmid species involved in 2,4-D degradation is being sought by the isolation and characterisation of plasmid DNAs from JMP116 and JMP119.

Torstensson *et al.*[8] have reported that the continued application of 2,4-D results in a progressive decrease in its persistence. They point out that the adaption hypothesis does not explain why this increased ability to degrade 2,4-D persists from one year to the next. Our results show that the presence of pesticide degrading plasmids have an important role in the lack of persistence of 2,4-D. This report of a pesticide degrading plasmid and its relationship

Table 1 The relationship between the inheritance of a conjugative plasmid and the ability to degrade 2,4-D

Possible phenotypes	No. of colonies
Ped$^+$ φ^r	0
Ped$^+$ φ^s	52
Ped$^-$ φ^r	1,000
Ped$^-$ φ^s	0

Ped$^{+/-}$, ability/inability to degrade 2,4-D. $\varphi^{r/s}$, Resistance/sensitivity to the plasmid specific bacteriophage PR11. Intrastrain mating between JMP116 (*Ped*$^+$) and a nalidixic acid-resistant mutant of JMP119 (*Nal*^{r} *Ped*$^-$). Exponential (about 1×10^9 CFU ml^{-1}) cultures of the donor (JMP116) and the recipient (JMP119 *Nal*^{r}) were diluted one-tenth in PYE broth, mixed in equal volumes and incubated without aeration at 32 °C overnight) (18 h). The recipient was re-isolated on PYE agar containing 20 µg ml^{-1} nalidixic acid. The re-isolated recipient clones were tested for their susceptibility to the plasmid-specific bacteriophage PR11 and ability to degrade 2,4-D in Loos' media[3].

to RP1 raises a number of important questions. How widespread are pesticide degrading plasmids and to what extent do they contribute to the degradation of the myriad of man-made pesticides in use today? Are pesticide-degrading plasmids analogous to antibiotic-resistant plasmids in that the application of the pesticides elicits an epidemic spread of the plasmid through the soil microbial population?

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An antiviral substance extracted from *Streptococcus faecalis*

ALTHOUGH there are a number of reports on virus-inhibiting substances, knowledge of those of bacterial origin is rather limited[1–6]. Horsfall and co-workers[7,8] reported that polysaccharides of *Klebsiella pneumoniae* suppressed multiplication of pneumonia virus of mouse *in vivo* and of mumps *in ovo*. Carver *et al.*[9–11] described virus-inhibiting factor(s) which they isolated from different species of bacteria belonging to both Gram-positive and Gram-negative groups. We report here that a substance or substances extracted from *Streptococcus faecalis* inhibited proliferation of certain human pathogenic viruses *in vitro* and *in vivo*.

S. faecalis organisms cultivated in VF bouillon supplemented with 1% glucose and 0.04% cystine were washed and sonicated (20 KHz, 30 min). Supernatant fluid obtained by a centrifugation (12,000*g*, 30 min) was the crude extract; the protein content was determined by the method of Lowry *et al.*[12]. Test virus was usually herpes simplex virus type 1 (HSV-1) propagated in HeLa-S$_3$ cell cultures, and in addition adenovirus type 12 (Ad-12) grown in KB cells.

The HSV suspension and the extract were simultaneously introduced into HeLa monolayer cultures in bottles and incubated at 37 °C for 1 h to allow complete cell–virus adsorption. After removing the excess fluid, overlay medium consisting of methylcellulose (0.5%), calf serum (3%) and kanamycin (60 µg ml^{-1}) in Eagle's minimum essential medium was added. Following incubation at 37 °C for 4 d, the cultures were stained with 1% crystal violet in ethanol. Numbers of plaques thus formed were significantly reduced than those in control cultures added with 0.01 M phosphate-buffered saline (PBS, *p*H 7.0) (ref. 13) in place of the bacterial extract.

The crude extract was subjected to salting out by ammonium sulphate and to acetone treatment, then gel filtration with Sephadex G-100. Particular fractions were comparatively effective in the plaque-inhibiting capacity; typical examples are shown in Fig. 1.

The antiviral substance (AVS) also exhibited an *in vivo* activity. AVS and HSV-1 were simultaneously injected into mice intraperitoneally (i.p.) and mortality ratios of AVS-treated mice were significantly lower than those of control mice given virus alone (Table 1).

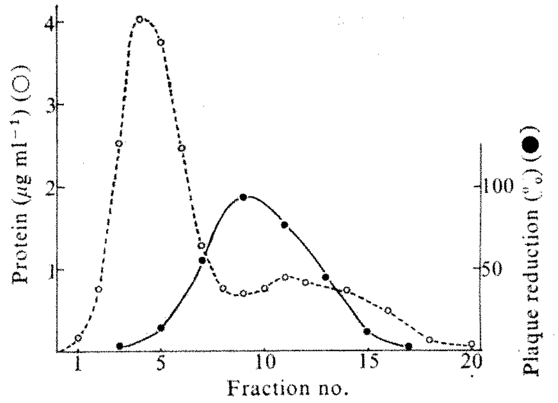

Fig. 1 Profile of Sephadex G-100 gel filtration of an ammonium sulphate precipitate of *Streptococcus faecalis* extract. The bacterial extract was prepared by the procedures indicated in text and adjusted to contain 15 mg ml^{-1} protein by addition of 0.01 M PBS (*p*H 7.0). Ammonium sulphate was then added to 70% saturation and stirred for 30 min at 5 °C. After a centrifugation at 15,000*g* for 30 min, the supernatant was brought to 90% saturation of $(NH_4)_2SO_4$ and stirred for 30 min at 5 °C. The precipitate collected by centrifugation (15,000*g*, 30 min) was dissolved in PBS and dialysed against PBS for 24 h at 5 °C. The non-dialytic fluid was adjusted to contain 8 mg ml^{-1} protein with PBS and gently added with acetone to 40% concentration, which was placed at −10 °C for 20 min. Following a centrifugation (10,000*g*, 30 min), acetone was added to the supernatant to a final concentration of 45% and again placed at −10 °C for 20 min. The precipitate collected by centrifugation (10,000*g*, 30 min) was then dissolved in PBS and dialysed against PBS at 5 °C for 24 h. After the non-dialytic fluid was adjusted to contain 40 mg ml^{-1} protein with PBS, the solution (5 ml) was charged on a Sephadex G-100 column (26 × 700 mm, Pharmacia, previously equilibrated with PBS) and eluted with PBS. Fractions (6 ml each) were collected and each of them was adjusted to contain 800 µg ml^{-1} protein. One-tenth ml each of the samples and 0.1 ml of HSV-1 suspension (adjusted to contain 100 PFU) were simultaneously introduced into HeLa cell monolayer cultures to determine plaque titres.

Similar activity was seen in experiments using suckling hamsters infected with Ad-12 virus. Development of virus-induced tumours was suppressed by administration of AVS. The effect was unequivocal when the AVS was given simultaneously with the virus, but the same substance given 15 d after virus inoculation was much less active (Table 2).

AVS (300–1,000 µg protein per ml) had no direct effect on the infectivity of viruses suspended in culture medium or PBS, and showed no acute toxicity to HeLa cells (at least as determined by morphology under an ordinary light microscope and proliferation capacity in bottles). Injection of AVS i.p. into 3-week-old white mice (320 μg as protein per mouse once a day for 10 days) brought about no loss of body weight, compared with control untreated mice. The antiviral activity resisted heating at 95 °C for 5 min or treatment with RNase or lipase, while it was completely lost or significantly reduced by treatment with proteolytic enzymes such as pronase or trypsin. The AVS was non-dialytic. These data suggest that the active principle is a protein.

AVS apparently lacked activity as an interferon stimulus. Mice, aged 3 weeks, were injected i.p. with AVS (60–1,500 μg protein in 0.4 ml PBS, per mouse). Plasma was taken after 3, 6 and 24 h and interferon titres were determined by the method of Younger *et al.*[14] using vesicular stomatitis virus (VSV) and mouse L cells. No interferon induction was noted in any of the test systems. We also found that the VSV-susceptibility of L cells pretreated with AVS was practically the same as that of control untreated cells; no difference of VSV titres was noted between these two cellular systems.

Table 1 Curative effect of AVS on HSV-1 infection in mice

	Mortality ratio of mice		
Group of mice	Experiment I	Experiment II	Experiment III*
AVS-treated	5/30 (17)†	5/23 (22)	14/85 (16)
Control	24/30 (80)	19/24 (79)	66/85 (78)

Three-week-old male mice (ddY-F strain) were each injected with HSV-1 i.p. (2,000 plaque-forming units each) and AVS (320 μg protein) simultaneously. Mice in control groups received the same virus alone. Observation periods ended at 14 d after the injection. Differences of mortality ratios between the AVS-administered and control groups are significant at the confidence level of 95%.

*Summary of 11 separate experiments carried out in the same way.

†Numerator indicates number of mice which died showing typical signs of HSV-1 infection, and denominator indicates number of mice inoculated; figure in parenthesis indicates percentage.

Some of the mechanism(s) underlying the phenomena were investigated as follows. The HeLa cell monolayer cultures were given HSV-1 and AVS simultaneously. After being incubated at 37 °C for 1 h, the viral titres of fluid phase were determined. As shown in Fig. 2, the amounts of virus which remained unadsorbed in the fluid of AVS-administered cultures were significantly higher than those of the same virus present in the fluid of control cultures receiving PBS in place of the AVS. It was also noted that the amounts of unadsorbed virus were almost constant regardless of the titres of original virus inocula, both in the AVS-administered and control cultures. It seemed that the AVS affected the virus at the stage of adsorption on to host cells.

Extracts were prepared from other kinds of bacteria and tested for virus-inhibiting capacity as above. In all 19 strains of organism of 17 species, including *S. lactis, Lactobacillus acidophilus, L. casei, Bifidobacterium bifidum, Escherichia coli* and *Proteus vulgaris* were examined. None of these samples was superior to the AVS from *S. faecalis* in the antiviral effects. It therefore seems that the virus-inhibiting activity described here may be species-specific.

Table 2 Inhibitory effect of AVS on development of tumours induced by Ad-12

Inoculum*	Tumour incidence (%)	P§
Experiment I		
Ad-12† plus AVS‡ simultaneously	9/19 (47.3)	< 0.01
Ad-12 plus AVS 15 days later	17/20 (85.0)	> 0.2
Ad-12 alone	19/20 (95.0)	
Experiment II		
Ad-12 plus AVS simultaneously	4/11 (36.4)	< 0.05
Ad-12 plus AVS 15 days later	10/11 (90.9)	> 0.2
Ad-12 alone	11/12 (91.7)	

*Inoculated subcutaneously in the back of 1-day-old hamsters.

†5×10^6 $TCID_{50}$ (tissue culture infecting dose) in 0.05 ml; Huie strain grown in KB cells.

‡0.265 mg protein in 0.05 ml.

§Calculated by the χ^2 test.

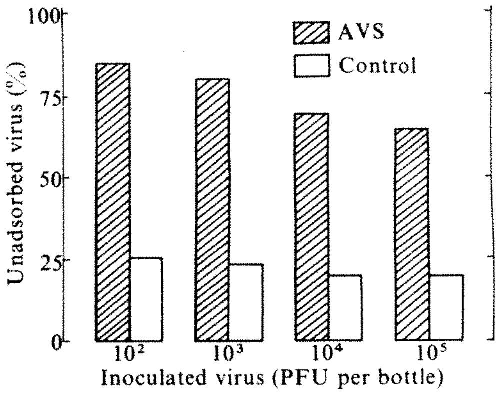

Fig. 2 Amounts of HSV-1 remaining unadsorbed in the fluid phase of HeLa-S_3 monolayer cultures receiving simultaneously virus plus AVS or virus plus PBS. HSV-1 (titres as indicated in abscissa) and AVS (160 μg protein) were simultaneously introduced on to HeLa monolayer cultures in bottles. Control cultures received the same virus plus PBS instead of the AVS. After incubation at 37 °C for 1 h, excess fluid was taken and assayed for the plaque titres which were taken to indicate amounts of virus unadsorbed. PFU, plaque-forming units.

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An immunological basis for inhibition of transformation of human lymphocytes by EB virus

IT IS well known that Epstein–Barr virus (EBV) transforms human lymphocytes *in vitro*, and lymphoblastoid cell lines are established[1]. Macrophages enhance transformation by EBV *in vitro*[2] but may not be essential[3]. Inhibition of transformation by EBV *in vitro* has also been reported, and was first observed when lymphocytes from an adult donor with antibody to EBV were infected with EBV and co-cultivated with autochthonous fibroblasts derived from skin[4]. Fibroblasts of foetal origin did not have this inhibitory effect. Further studies established the principle that co-cultivation of EBV-infected lymphocytes with autochthonous or allogeneic fibroblasts of adult but not foetal origin, was associated with inhibition of transformation by EBV (ref. 5). This report presents evidence that this type of inhibition of transformation by EBV is specifically mediated by mononuclear cell preparations from donors previously infected with EBV. An immunological basis is highly probable.

The major characteristics of the experimental system for studying inhibition of transformation by EBV are noted briefly. First, inhibition is defined as the failure of non-adherent lymphocytes to aggregate and proliferate yet still remain infected and viable. Second, inhibition has not been shown to be due to a factor released by adult fibroblasts. Third, inactivation of the inoculated EBV is not the cause of inhibition because an EBV-associated nuclear antigen (EBNA) is demonstrable in the inhibited lymphocytes for several weeks[5]. Fourth, the inhibition is reversible up to at least four weeks after inoculation of EBV by simply adding phytohaemagglutinin to the cultures[6] or by removing the non-adherent lymphocytes from the adult fibroblasts and transferring them to a clean tissue culture vessel[5,6]. Finally, reversal of inhibition results in proliferation of the EBV-infected cells within a few days, and typical lymphoblastoid cell lines emerge[5]. The proliferation of cell lines already transformed by EBV is not inhibited in the presence of adult fibroblasts[5].

In a series of experiments, inhibition of transformation by EBV was consistently recorded when EBV-infected lymphocytes of three donors with antibody to EBV were co-cultivated with fibroblasts from skin of two adult donors, but not when they were co-cultivated with fibroblasts derived from four different foetuses. In contrast, inhibition of transformation by EBV was not demonstrated with lymphocytes from a donor without antibody to EBV. This observation prompted a detailed investigation of the relationship between inhibition of transformation by EBV *in vitro* and the previous history of EBV infection of the lymphocyte donor.

The methods used in this work have been described in detail[1,7]. In essence, mononuclear cell populations were prepared from peripheral blood and the adherent cells removed by incubation in medium containing autochthonous serum in plastic Petri dishes. The non-adherent mononuclear cell population (mainly lymphocytes) was infected with filtered EBV of the QIMR–WIL strain and cultured in microtitre plates. Appropriate fibroblast cultures were usually prepared on the previous day. The cultures (at least six replicates) were maintained in medium RPMI 1640 containing 16% inactivated foetal calf serum and observed microscopically for evidence of transformation for at least 4 weeks, although transformation was usually evident in 7 d.

The results of investigations on 14 donors (10 males and 4 females, aged 5–46 yr) were quite clearcut. Non-adherent lymphocytes from each donor, whether with or without antibody to EBV, were uniformly transformed in the usual manner by EBV when cultivated alone or with foetal fibroblasts. A strikingly different pattern occurred in the presence of adult fibroblasts; transformation occurred uniformly with lymphocytes from all eight donors (six adults and two children) without antibody to EBV but with none of the lymphocyte samples from six donors (five adults and one child) with antibody to EBV. In addition, lymphocytes from four umbilical cord blood samples transformed as usual on adult fibroblasts.

In the work reported here, the lymphocyte concentration was 0.5×10^6 ml^{-1} and the multiplicity of EBV infection (MOI) 0.1 TD_{50} per cell. Inhibition was also observed with a MOI of 15 and a cell concentration ranging from 10^5–10^6 ml^{-1}, showing that the phenomenon was not dependent on the dose of virus or the cell concentration. Two experiments were carried out in a different manner. Non-adherent lymphocytes from donors without previous EBV infection (adult and cord) were infected with EBV, mixed with uninfected lymphocytes from donors with previous EBV infection, and co-cultivated with adult fibroblasts. In these conditions, viral transformation was inhibited as described above, confirming that inhibition of transformation is dependent on the presence of lymphocytes from EBV-immune donors.

The effect of previous EBV infection on the role of adult fibroblasts in the inhibition of EBV transformation was also studied. The results, summarised in Table 1, showed that inhibition was dependent on previous EBV infection of the donor of the lymphocytes but not of the donor of the adult fibroblasts.

From the present results it now seems likely that an immunological system capable of inhibiting transformation of lymphocytes by EBV is inherent in the mononuclear cells from persons with previous EBV infection. Detection of this inhibition is so far dependent on particular culture conditions, and the role of fibroblasts of adult origin seems to be simply to provide these conditions. Failure of non-adherent mononuclear cells to inhibit transformation when cultivated alone or in the presence of fibroblasts of foetal origin may be due to unsuitable culture conditions or to interference by other cellular factors. The mechanism of inhibition is unknown but it may well involve a complex interaction of various immunological components. These include the expression of EBV antigens on infected target cells, the particular EBV-specific immunological effector system involved, the survival and activity of the components of the effector system in various culture conditions, and the influence of various mitogenic factors. Obvious possibilities are that the inhibition involves T lymphocytes, antibody, or antibody-mediated cellular immunity, acting at the surface of the EBV-infected cells to modulate their behaviour. These aspects are being examined.

Table 1 Effect of previous EBV infection on role of fibroblasts during inhibition of EBV transformation

Leukocyte donor and reciprocal antibody titre to viral capsid antigen	Fibroblast donor and reciprocal antibody titre to viral capsid antigen	Proportion of cultures transformed
P.G.P. (< 10)	S.G.B. (160)	6/6
	B.D.B. (< 10)	6/6
	Foetal (NT)*	6/6
	None	6/6
K.D. (< 10)	S.G.B. (160)	6/6
	B.D.B. (< 10)	6/6
	Foetal (NT)	6/6
	None	6/6
S.G.B. (160)	S.G.B. (160)	0/6
	B.D.B. (< 10)	0/6
	Foetal (NT)	6/6
	None	6/6
D.J.M. (40)	S.G.B. (160)	0/12
	B.D.B. (< 10)	0/12
	Foetal (NT)	12/12
	None	12/12
Umbilical cord (NT)	S.G.B. (160)	12/12
	B.D.B. (< 10)	12/12
	Foetal (NT)	12/12
	None	12/12

*NT, Not tested.

Inhibition of transformation by EBV without death of the target cells contrasts with the cytotoxity for EBV-infected target cells of selected lymphocyte populations from patients with acute infectious mononucleosis[8–9]. Lymphocytes from persons with longstanding EBV infections were not cytotoxic in those two systems. The specificity of inhibition of transformation contrasts also with the nonspecific inhibition by human lymphocytes of EBV lymphoblastoid cell lines[10–12], providing further support for the view that inhibition of EBV transformation acts at a point before the process is complete.

The manner in which EBV persists following infectious mononucleosis is of considerable interest. It has been suggested that cells transformed by EBV persist in the body probably under immunological control[13], or that the EBV genome may persist in cells in an incompletely expressed form[14]. The present studies of inhibition of transformation bear on this particular problem as well as on wider questions of viral latency, especially with herpesviruses.

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Intermittent exposure to fluorescent light extends lifespan of human diploid fibroblasts in culture

SHORT exposure (3 or 4 h) to fluorescent light one to three times weekly enhances the proliferation rate of human diploid fibroblasts in culture[1]. This proliferative response is reversible and is mediated through the culture medium, Dulbecco Vogt's supplemented with 10% foetal bovine serum. Conceivably, this increase in proliferation rate might also influence the lifespan of human diploid fibroblasts, which undergo a limited number of population doublings (PDs) in culture[2]. To evaluate the long term effects of repeated light exposure on lifespan, three lines of human fibroblasts originating from adult skin (HU-274), a mince of whole foetus (HU-278), and foetal lung (WI-38), were assayed in quantitative experiments to determine the lifespan measured as cumulative cell number and total PDs

Irrespective of the PD level of the cells at the beginning of the experiment, repeated exposure (3 h) to cool white fluorescent light (6 to 9 W m^{-2}) three times weekly, reproducibly extended the lifespan. This extension was reflected by an increase in the total number of PDs, cumulative increase in cell number, and duration of cell survival and growth (Figs 1–3). Cessation of growth was indicated by a decrease in cell number from inoculum size after 2 weeks' incubation. The cells of both foetal lines, WI-38 and HU-278, which before this experiment had undergone 44 or more PDs and had been exposed to room fluorescent lights during routine handling, showed a 19–21% increase in total PDs (Table 1). In contrast, the cells of adult origin, HU-274, which had never been exposed to light of wavelength less than 500 nm during the initial seven PDs in culture showed a 70–98% increase in total PDs. The unexposed cells of line HU-274 underwent a total of 20–25 PDs before cessation of growth. Cells of the exposed lines, on the other hand, have undergone 40–42 PDs to date, and proliferation rates have decreased to a level of 1.5–1.7-fold increase over a 4-week period. Light exposures have been reduced to two per week, and the cultures are being maintained to determine if they can become established as continuous cell lines.

Although the proliferation rate of human fibroblasts in culture may be increased by a number of environmental factors including the volume of culture medium per cell[3], serum concentration[3], medium formulation (R. Parshad, unpublished). and intermittent light exposure[1], the only factor shown to increase proliferation rate as well as extend the lifespan reproducibly is the addition of cortisone or hydrocortisone to the medium[4]. The addition of hydrocortisone to the medium of W1–38 cells increased the PDs 30–40% above those of untreated controls. The duration of

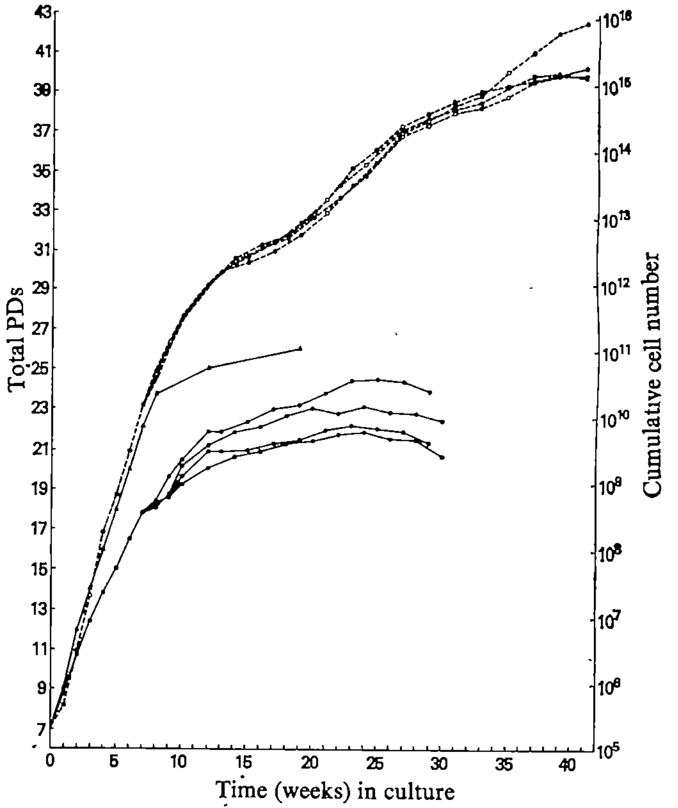

Fig. 1 Effect of intermittent light on lifespan of human skin fibroblasts, line HU-274, derived from a 50-yr-old black male who died of hypertension and renal failure. The cells grown in Dulbecco Vogt's medium supplemented with 10% foetal bovine serum had been carried for seven PDs as estimated from 1:2 or 1:4 split ratios before this experiment. In the quantitative studies, 2×10^5 cells were inoculated into 5 ml medium in a T-25 flask (Falcon). Light-exposed cultures were illuminated under two cool-white fluorescent light tubes (GE F 15T8/CW) at 27 cm from the floor of the flask with an exposure intensity of 6 to 9 W m^{-2} for 3 h, three times a week, 24 h after inoculation and twice thereafter following culture fluid renewal. After an interval of 1 or 2 weeks, depending on cell growth, the number of cells per flask was determined by haemocytometer. Cells from replicate cultures were inoculated at 2×10^5 cells per flask to continue the line and quantify as above. The PDs for the shielded, non-quantified cell line are based on 1:2 or 1:4 split ratios. Each point is the average of counts from four replicate cultures. Except for the light exposure described above, cells and culture medium were handled under red or gold light and were never exposed to light of wavelength below 500 nm. ●, Unexposed; ○, light exposed

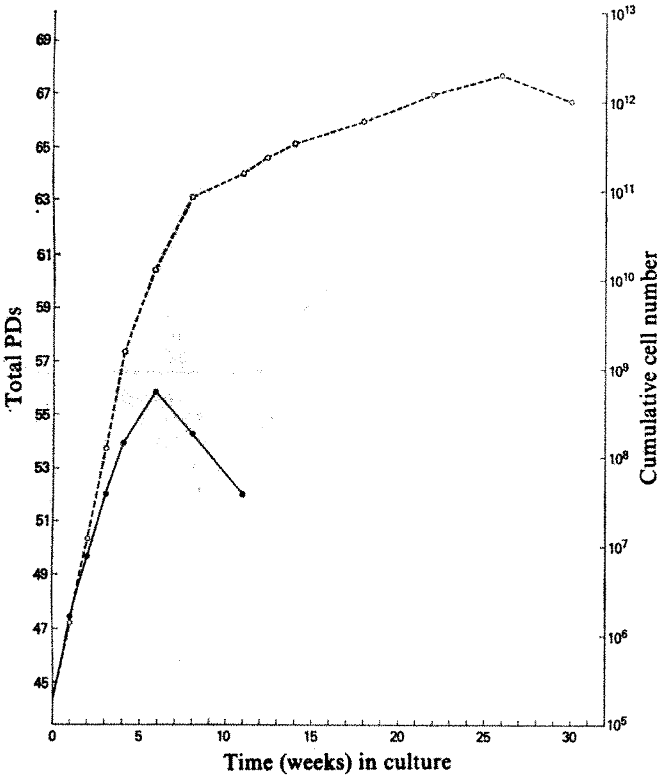

Fig. 2 Effect of intermittent light on lifespan of foetal lung WI-38 cells obtained at passage 17 from the American Type Culture Collection Rockville, Maryland. Before this quantitative experiment, cells had been carried in NCTC 135 with 10% foetal bovine serum for 21 PDs and in Dulbecco Vogt's medium for six PDs based on 1:4 or 1:8 split ratios. For experimetal details, see Fig. 1. ●, Unexposed; ○, light exposed.

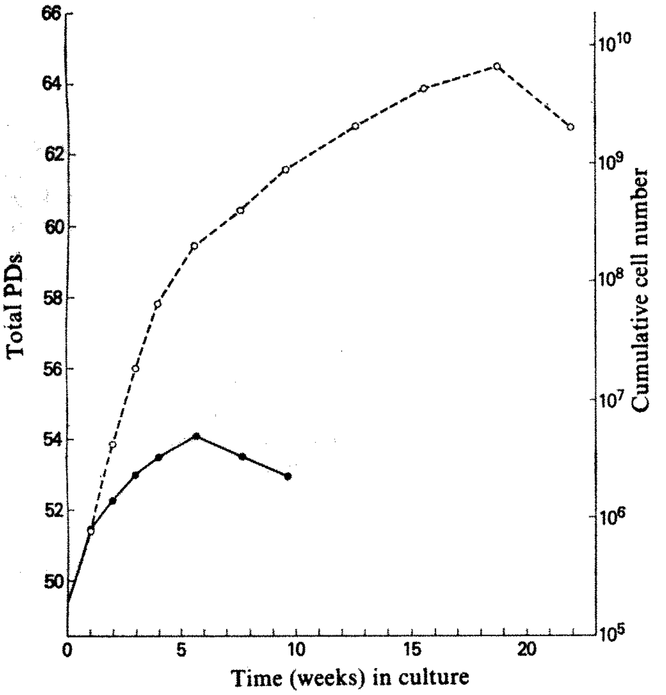

Fig. 3 Effect of intermittent light on lifespan of human foetal cells, line HU-278, derived from a mince of whole foetus. Before this quantitative experiment cells had been carried in NCTC 135 with 10% foetal bovine serum for 38 PDs before cryopreservation, and for 11.5 additional PDs in Dulbecco Vogt's medium based on 1:4 or 1:8 split ratios. For experimental details, see Fig. 1. ●, Unexposed; ○, light exposed.

Table 1 Effect of intermittent light exposure on lifespan (PDs of) human fibroblasts in culture

Cell line	Initial PD before light exposure	Additional PDs* Unexposed	Additional PDs* Exposed	% Increase in exposed to total unexposed PD†
HU-274-1	7	17.53	34.79	70.40
-2	7	15.07	32.73	80.02
-3	7	14.55	32.70	84.22
-4	7	12.56	32.76	98.02
HU-278	49.5	4.66	15.04	19.17
W1-38	44.5	11.44	23.25	21.11

*PD (x) was calculated by the equation $N = N_0 2^x$. Where N_0 = initial cell number and N = final cumulative cell number.

†PD of exposed − PD of unexposed ÷ Initial PD + PD of unexposed × 100.

life in culture for both control and treated cells, however, was the same. Intermittent light treatment, here, of W1–38 cells increased the PDs 100% above those of unexposed controls. When intermittent light exposure of adult skin cells (HU-274) was started early in culture, the maximum increase in PDs was 160% above those of unexposed controls. In all three cell lines, the duration of life in culture was also greatly increased.

Although cytotoxic effects of fluorescent light on cells in culture have been reported[5], Litwin[6] observed an increased lifespan but no enhanced proliferation rate in human embryonic fibroblasts when exposed to light for 2 or 4 h daily. In the conditions of the present study, which include a relatively high inoculum size, low light intensity, and short duration of exposure, stimulation of proliferation rate and extension of lifespan were observed in all three cell lines of adult or foetal origin. We have shown previously that the stimulation of proliferation by fluorescent light is mediated through the culture medium. The extension of lifespan may likewise be associated with photochemical changes in some component(s) of the medium.

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Contact-mediated communication of ouabain resistance in mammalian cells in culture

THE active transport of Na^+ and K^+ across the plasma membrane, mediated by Na^+-K^+-ATPase, can be inhibited specifically by the cardiac glycoside ouabain[1]. Ouabain is toxic to mammalian cells in culture, the degree of sensitivity varying with species[2]; however, cells resistant to its cytotoxic effects have been obtained in rodent[2–4] and human[5,6] cell strains. Ouabain-resistant (oua^r) cells are thought to have a mutation affecting the Na^+-K^+-ATPase so that their enzyme has a lower affinity for ouabain than the enzyme of ouabain-sensitive (oua^s) cells. Therefore, oua^r cells can actively transport Na^+ and K^+ at ouabain concentrations that would inhibit oua^s cells[2,4,5]. We have observed that oua^s human cells, when cocultured densely with oua^r human cells, acquire a functional resistance to ouabain as evidenced by their ability to proliferate in medium containing the drug (unpublished observations). We suspected that ouabain resistance was communicated from oua^r to oua^s cells and that this transfer was a manifestation of ionic transport through a pathway independent of the Na^+-K^+-ATPase. There is substantial evidence that a specialised membrane structure, the gap junction, is the ultrastructural pathway for the transfer of ions, metabolites and other small molecules between cells in contact[7–9]. To determine whether the acquisition of ouabain resistance we observed was mediated through gap junctions, we have compared—for the ability to communicate ouabain resistance—cells which are known to form gap junctions with those that cannot. Our results indicate that ouabain resistance can be communicated from oua^r to oua^s cells, that the process is contact dependent and that it does not occur in cells unable to form gap junctions.

To assay for communication of ouabain resistance, we analysed chromosome preparations obtained from mixed cultures of oua^r and oua^s cells in ouabain for the presence of metaphase cells of oua^s origin. The ability of oua^s cells to divide in ouabain would provide definitive evidence that the sensitive cells had acquired resistance We used mouse fibroblasts as the oua^r cells and human fibroblasts as the oua^s cells for the following reasons. Mouse and human metaphases are distinguishable by conventional staining techniques (Fig 1). Mouse cells are naturally 10^4-fold more resistant to ouabain than human cells, so they can proliferate in 10^{-6} M ouabain, whereas human cells cannot. There are mouse cell strains that form gap junctions, and others that do not. The oua^r cells were therefore (1) mouse 3T3 fibroblasts, which are known to be communicating because they form gap junctions[10], ionically couple[11] and transfer nucleotides[12], and (2) mouse L fibroblasts known to be non-communicating because they do not form gap junctions[7,8] and do not transfer ions[7,8] or nucleotides[7,8,12–14]. The oua^s cells were a strain of human skin fibroblasts (no. 106) isolated in our laboratory from a 2-yr-old female, they were used in their 5–8th subculture and have been shown to communicate nucleotides[6,14] Human skin fibroblasts are known to form gap junctions and transfer ions[8].

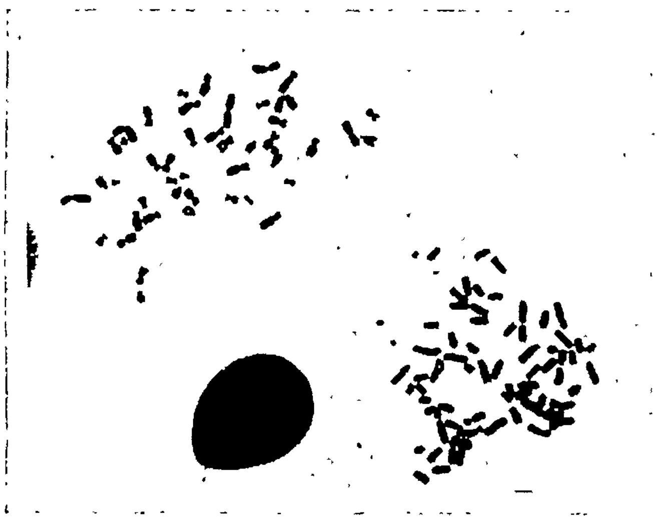

Fig. 1 Photomicrograph showing human and mouse metaphases from mixtures of human and 3T3 cells in 10^{-6} M ouabain Chromosome preparations were made *in situ*: the cells on 22-mm² coverslips were exposed to colchicine (0.4 μg ml^{-1}) for 1 h, 0 075 M KCl for 5 min, and 3.1 methanol·acetic acid for 30 min at 4 °C Coverslips were then immersed in ice-cold water, dried on a hot plate and stained with Giemsa. A clone of 3T3 cells with very few metacentric chromosomes was used to facilitate discrimination of the metaphases as either mouse or human. (× 663.)

Table 1 Percentage human metaphases of total metaphases in cultures of human and mouse cells plated into control and ouabain medium

Experiment	Cells	Ouabain conc in medium	Total metaphases	% Human metaphases
1	Human	0	200	100
		10^{-6} M	0	0
	Human+L	0	200	45
		10^{-6} M	200	0
	Human+3T3	0	200	54
		10^{-6} M	200	51
2	Human	0	200	100
		10^{-6} M	0	0
	Human+L	0	200	50
		10^{-6} M	200	0
	Human+3T3	0	200	55
		10^{-6} M	200	49

For experiment 1 cells were plated on to 22-mm² coverslips in 35-mm dishes containing 2 ml of medium and incubated at 37 °C in 5% CO_2. After 24 h of cultivation, the medium was renewed to ensure stringent selection. After 48 h, coverslips were fixed and stained for chromosome analysis as described in Fig 1 For experiment 2 cells previously plated on to 22-mm² coverslips were transferred to the same 100-mm dish (Fig 2) and then treated as in experiment 1. In both experiments cells were plated at the same densities—10^5 human cells and 5×10^4 mouse cells per 35-mm dish Cells were maintained in growth medium (Eagle's minimal essential medium supplemented with non-essential amino acids, glutamine, penicillin-streptomycin, fungizone and 20% foetal calf serum (Gibco)) Growth medium containing 10^{-6} M ouabain is referred to as ouabain in the text. Human metaphases were distinguished from mouse at ×100 magnification

The human cells were plated alone or co-cultivated with either L or 3T3 cells at densities ensuring cell contact[13,14] in medium containing 10^{-6} M ouabain (hereafter referred to as ouabain). For a control, a similar series was plated into non-selective medium The cultures were incubated, as described in Table 1, and then chromosome preparations were made. Analysis of the metaphase spreads (Table 1, experiment 1) revealed, as expected, that L and 3T3 cells divided in ouabain, whereas human cells, when plated alone, did not In the mouse–human cell mixtures plated into ouabain, no human metaphases were found when human cells were co-cultivated with L cells; however, abundant human metaphases were observed when human cells were co-cultivated with 3T3 cells (Fig. 1). Thus, human cells were able to acquire ouabain resistance from 3T3 cells but not from L cells.

To assure that the communication of ouabain resistance observed in the 3T3–human cell mixtures was not attributible to passage of molecules through the medium, the experiment was repeated in the following conditions. Coverslips containing the cell mixtures and appropriate controls were transferred to the same large Petri dish containing either ouabain or non-selective medium (Fig 2) The results of metaphase analysis from this experiment (Table 1, experiment 2) were identical to those of experiment 1 in which cells had been incubated in individual Petri dishes. The human cells on the same coverslip with 3T3 cells were able to divide in ouabain, whereas human cells in the same dish, but not on the same coverslip with 3T3 cells, were unable to divide. The absence of human metaphases on coverslips containing human cells alone or in combination with L cells not only confirms that L cells cannot transfer ouabain resistance, but also indicates that the communication of ouabain resistance seen with 3T3 cells does not occur through the medium. Moreover, increasing the ratio of 3T3 to human cells (by floating a 22-mm² coverslip containing human cells on a confluent lawn of 3T3 cells in a 100-mm dish) did not enable the human cells to divide in ouabain (data not shown). The best interpretation for our observation is that the communication of ouabain-resistance is dependent on contact between human and 3T3 cells.

Our results demonstrate that L cells, characterised by an inability to form gap junctions, cannot confer ouabain resistance on oua^s cells, whereas 3T3 cells, which are able to form gap junctions, can transfer ouabain resistance This provides compelling evidence that the gap junction mediates the communication of ouabain resistance and also suggests a molecular basis for this communication. There is substantial evidence from experiments

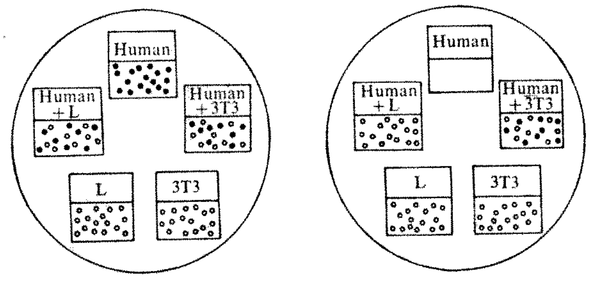

Fig. 2 Experiment demonstrating that communication of ouabain-resistance does not occur through the medium. Mouse and human cells, as indicated, were plated on to 22-mm^2 coverslips in individual 35-mm dishes containing non-selective medium. After cell attachment (5 h), the coverslips were transferred into a 100-mm dish containing 10 ml of non-selective (*a*) or ouabain (*b*) medium and treated as described in Table 1, experiment 2. The results of metaphase analysis are indicated schematically: ●, human metaphases; ○, mouse metaphases. In ouabain only the human cells on the same coverslip with 3T3 cells are able to divide.

involving both ultrastructural and electrophysiological techniques that Na^+ and K^+ can diffuse freely from cell to cell through the low-resistance pathways of gap junctions without leakage into the extracellular space[7–9,11,15–17]. Diffusion of Na^+ and K^+ through gap junctions between oua^r and oua^s cells would maintain the correct internal cationic balance in the oua^s cell, even though its own Na^+-K^+-ATPase is inhibited by ouabain.

Alternative explanations, for example, transfer of resistant Na^+-K^+-ATPases through gap junctions, are unlikely because these junctions are not permeable to molecules greater than 1,200 daltons[18]. The large proportion of responding cells precludes a rare event such as spontaneous mutation as a cause; in fact, we have not observed spontaneous mutations to ouabain resistance in 5×10^7 human skin fibroblasts[6]. Nor does the oua^r phenotype significantly affect the ability of a cell to communicate[6]. One cannot exclude the possibility that resistant Na^+-K^+-ATPases are transferred to the sensitive cells through focal membrane fusion, or diffusion in the immediate environ of resistant cells, but neither of these nor other mechanisms readily explain the observed differences between 3T3 and L cells. On the basis of our results, the most likely explanation is that resistance is transferred by diffusion of ions through gap junctions.

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Synergism between anti-microtubule agents and growth stimulants in enhancement of cell cycle traverse

COLCHICINE and other mitotic inhibitors have been used widely in cell cycle studies to produce synchronous cell populations[1]. This application is based on the ability of these drugs to inhibit mitosis by binding to tubulin and preventing microtubule (mitotic spindle) formation[2]. As well as inhibiting mitosis, these drugs modify the morphology and functions of cells during interphase. For example, colchicine inhibits the formation of cilia[3], affects phagocytosis, the normal distribution of lysosomes[4], and the synthesis and secretion of various proteins[5–7] and alters the transport of certain nucleotides[8]. At very high concentrations ($2–35 \times 10^{-3}$ M) colchicine and colcemid retard the initiation of DNA synthesis and prolong the period of DNA synthesis[9]. We describe here the synergistic effects of colchicine and other anti-microtubule agents at lower concentrations ($1–2 \times 10^{-7}$ M) in the insulin- or serum-induced increase in cells moving from G_1 to S phase. Our results suggest that microtubules have an important role in the regulation of DNA synthesis by growth factors.

High density chick cells starved of serum can be stimulated synchronously by insulin to traverse at least one cell cycle[10]. This stimulation was detected as an increase in the proportion of cells in S phase with 8–10-h lag when the increase in DNA per cell was detected by flow microfluorometry (FMF). When similar experiments were performed in the presence of 2×10^{-7} M colchicine, it was evident that colchicine significantly increased the population in the S phase of the cell cycle (Fig. 1). The stimulated cells progressed through S phase more slowly than in the absence of colchicine, but the degree of synchrony was as good as the stimulated population when insulin was used alone.

Two other anti-microtubule agents, colcemid and vinblastines were compared with colchicine in their ability to affect the proportion of cells stimulated to make DNA in insulin-treated chick cell cultures. All of these compounds increased the cell population moving out of G_1 phase in the presence of insulin (Table 1). Colchicine and vinblastine were more effective than colcemid at lower concentrations. Furthermore, the continuous presence of anti-microtubule agents was not necessary, for their removal from the media after 6 h had the same effect (data not shown). These observations suggest that the primary effect of anti-microtubule agents at low concentrations is on the early events in the simulation of DNA synthesis. At higher concentrations ($> 5 \times 10^{-7}$ M), all of these compounds decreased the number of cells engaged in DNA synthesis, probably due to toxic side effects. Thus the mode of action of these drugs is different when compared at low and high concentrations.

To determine whether or not this stimulatory effect also applied to cell lines, the effect of anti-microtubule agents on BALB 3T3 cells was compared with that on chick fibroblasts. In these experiments cultures at their saturation density were stimulated with fresh medium containing either insulin or serum. The proportion of cells in the $S+G_2+M$ phases with or without added growth stimulants are listed in Table 2. As before, we found that insulin had little or no stimulatory effect on DNA synthesis in BALB 3T3 A31 cells. This was true even when the insulin concentration was raised to 128 mIU ml^{-1}. Similar results were obtained with Don hamster cells at high density (results not shown). Furthermore, only chick cells could be stimulated to synthesise DNA by fresh medium. It is important to note that colchicine was effective in stimulating cell movement from G_1 to S only

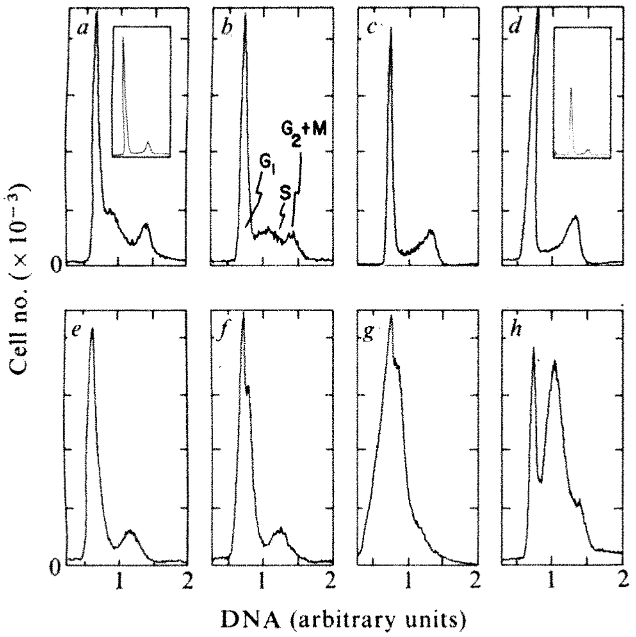

Fig. 1 Progress of chick embryo fibroblasts through the cell cycle in the presence of insulin and colchicine. Confluent secondary chick embryo fibroblasts in medium 199 were prepared and deprived of serum as described before[10]. After serum starvation, insulin was added at a concentration of 16 mIU per ml medium. Parallel cultures were provided with insulin and 2×10^{-7} M colchicine and were incubated in 5% CO_2 at 39 °C. Cells were collected and prepared for FMF analysis, using propidum iodide as the fluorescent stain[25]. FMF patterns of insulin-treated cultures are *a–d* for the time points of 13, 17, 23 and 29 h respectively, and insulin plus colchicine are *e–h* for the same time sequence. The inserted histograms in (*a*) and (*d*) are control cultures in medium 199 at 0 and 29 h. Histograms were plotted to give the peak channel at approximately the same height. The number of cells ($\times10^3$) in the peak channel was *a*, 2.7; *b*, 1.0; *c*, 1.3; *d*, 5.5; *e*, 4.0; *f*, 2.2; *g*, 4.0 and *h*, 3.5.

when the primary growth stimulant, a mitogen, was able to stimulate some cell cycle traverse. Therefore, colchicine and other anti-microtubule agents act as 'synergistic agents' to a 'mitogen'.

How does the message for DNA synthesis get transferred from the mitogen to the cell? The cell membrane is believed to have an important role in such progress. Some growth stimulants have been partially purified from various sources, and shown to bind to the surface membrane of cultured cells[11–13]. What occurs between the binding of growth stimulants and their relation to the initiation of DNA synthesis is, however, poorly understood.

The dynamics of the membrane receptors and other components seem to be subject to controls from both sides of the membrane. For example, concanavalin A (con A) and other related plant lectins inhibit the immunoglobulin capping on lymphocytes[14,15]. Con A by itself can form caps on various cell types and the process is either inhibited or stimulated by anti-microtubule or anti-microfilament agents depending on cell types studied[15–18]. But, the evidence for the involvement of microtubules in growth regulation has been of a 'negative' nature so far, that is, agents affecting these organelles would inhibit the stimulatory action caused by a mitogen[9]. Most of these studies were carried out at high concentrations of inhibitors, where possible toxic side effects could have complicated the interpretation of the results. Our evidence of the positive effect of anti-microtubule agents on mitogen-stimulated DNA synthesis directly demonstrates involvement of microtubules in growth regulation.

Table 1 Effect of colchicine, colcemid and vinblastine on cell cycle traverse of insulin-treated chick embyro fibroblasts

Concentrations of mitotic inhibitors (M)	% of cells in $S+G_2+M$ phases Colchicine	Colcemid	Vinblastine
0 (insulin only)	25	25	25
5×10^{-8}	47	27	58
1×10^{-7}	60	25	64
2×10^{-7}	69	54	60
5×10^{-7}	64	62	63

Cells were grown and prepared for FMF analysis as for Fig. 1. Colcemid, vinblastine, and colchicine were tested at the concentrations indicated. Insulin was present in all cultures. Cells were collected 22 h after addition of mitotic inhibitors and insulin. After FMF analysis, the % population in $S+G_2+M$ phases were calculated with a computer program.

The presence of insulin receptors on chick fibroblasts has been demonstrated in detail[19]. Furthermore, site–site interaction and redistribution of insulin receptors after the binding of insulin to the membrane of rat liver or human lymphocytes have been reported[20]. The striking synergistic effect of colchicine and insulin on stimulation of DNA synthesis in chick fibroblasts reported here makes it possible to propose the following model to explain such synergism (Fig. 2). In this model which is a modification of that proposed by Nicolson[21] and by McGuire and Barber[22], a growth stimulant after binding activates its receptor on the membrane by forming an active stimulant–receptor complex. To complete a signal for DNA synthesis, however, cooperation of this complex with certain membrane components must take place. If the mobility of membrane receptors and second components is an important factor in the signal transfer, any compound which increases this mobility should facilitate DNA synthesis. Microtubules have been proposed to be an anchorage agent for certain membrane components[14,21]. Our results indicate that this may indeed be the case. A factor which destroys microtubule structure, colchicine in this case, can be a synergistic agent for growth stimulation. Such an agent by itself is incapable of triggering DNA synthesis. It acts only when some DNA synthesis capacity is already present.

Table 2 The effect of colchicine and growth stimulants on cell cycle traverse

Cell types and media	% of cells in $S+G_2+M$ phases −Colchicine	+Colchicine
Secondary chick embryo fibroblasts		
Medium 199 (control)	14	32*
+insulin, 16 mIU ml⁻¹	29	66
+chick serum, 3%	33	58
BALB 3T3 A31		
Medium DME (control)	4	4
+insulin, 128 mIU ml⁻¹	4	4
+calf serum, 10%	15	29
+calf serum, 20%	30	78
+calf serum, 30%	33	90

Data for secondary chick embryo fibroblasts were obtained as described in Table 1. Values are averages of two experiments. BALB 3T3 A31 cells were grown to confluency in medium DME containing 10% calf serum for 5 d. Cells were washed with saline and provided with fresh medium DME containing additional components indicated. After 28 h, cells were collected and prepared for FMF analysis.

*The degree of stimulation on cells in medium 199 (control) varied with each experiment depending on the batch of cells.

It has been reported that BALB 3T3 cells indeed have insulin receptors[23]. But binding of insulin to these receptors fails to stimulate DNA synthesis in these cells (ref. 10, and the results here). In addition, the cell line of this type and human diploid foreskin or ovine embryonic trachea fibroblasts fail to show growth stimulation on addition of trypsin, an agent which stimulates growth in secondary

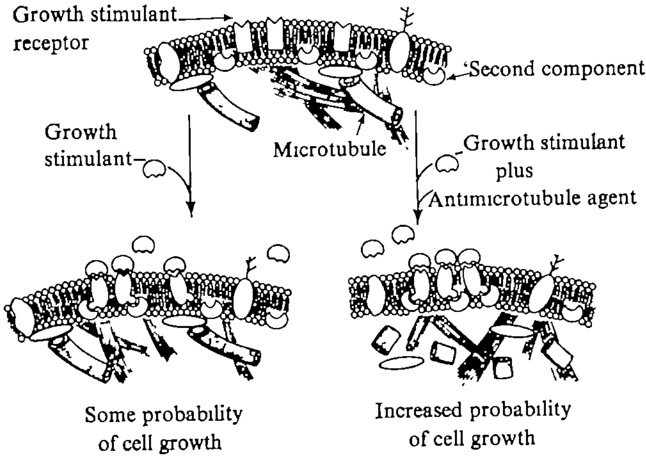
Growth stimulant receptor
'Second component'
Growth stimulant
Microtubule
Growth stimulant plus Antimicrotubule agent
Some probability of cell growth
Increased probability of cell growth

Fig. 2 Schematic representation of message transfer. The inactive receptor for 'growth stimulant (mitogen)' and 'second component' for carrying on message transfer are distributed randomly on the membrane, and their mobilities are controlled by membrane fluidity which in turn, is influenced by the cytoplasmic microtubules along the inner side of the membrane. When anti-microtubule agents are present, the cytoplasmic microtubules are depolymerised and the mobilities of membrane components increase. Thus, the chance of interaction between the activated receptor complex and the second component is higher and the message for DNA synthesis is transferred much more efficiently.

chick fibroblasts[24]. The most likely explanation is that there is fundamental difference in the nature of membranes of primary cell cultures and cell lines. It is possible that the interaction between mitogen receptor complex and second membrane component is blocked in cell lines unless a more complicated factor such as serum is provided to potentiate the cooperation. Alternatively, the activation of certain mitogen receptors, for example, insulin receptors, may not occur after the binding of the mitogen. Therefore, no message is generated and transferred to the second component. There is an additional possibility that the second membrane component for message transfer from particular single growth factor may be altered in cell lines. As a result, the growth control of cell lines in culture becomes much more stringent than that of primary cell cultures as discussed previously[10]. The exact nature of the synergistic effects of anti-microtubule agents and growth stimulants is currently under investigation.

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5-Hydroxytryptamine and punishment

PUNISHMENT is defined as the presentation of a noxious stimulus and results in the suppression of behaviour. This suppressive effect of punishment is seen clearly in conflict situations where the organism is encouraged to respond and then is punished for doing so. Brain 5-hydroxytryptamine (5-HT) systems have been implicated in the processes associated with the response to punishment. We show here that selective neurotoxin lesions to the 5-HT forebrain pathways reverse the suppressive effects of punishment, as do minor tranquillisers, supporting the view that 5-HT has a central role in conflict behaviour and in the effect of minor tranquillisers on such behaviour.

If a response is followed by punishment, the probability of its occurrence decreases. 5-HT antagonists[1-3] and synthesis inhibition[4-6] have been shown to re-instate rates of punished responding. Furthermore, behavioural release following synthesis inhibition has been shown to correlate closely with the pharmacological time course of 5-HT depletion[6]. Qualitatively similar behavioural effects have been reported with anxiolytic drugs such as the benzodiazepines and with pharmacological studies in support, it has been suggested that at least part of the anti-anxiety action of these drugs is due to decreased 5-HT functional activity[7]. But, the lack of neuropharmacological specificity associated with the existing methods for reducing 5-HT activity makes clear experimental investigation of this hypothesis difficult.

The neurotoxic agent, 5,7-dihydroxytryptamine can be injected directly into the brain to destroy 5-HT neurones. Stein and colleagues[8] have reported a profound, although transitory, release of punished behaviour in a conflict paradigm, accompanied by little effect or unpunished responding, following intraventricular injection of 5,6-dihydroxytryptamine (5,6-DHT). Neurotoxic indoleamines have been injected into specific brain areas[9] and the balance of neurochemical data (for instance ref. 10) suggests that 5,7-DHT produces minimal nonspecific degeneration. Therefore, intracerebral injections of 5,7-DHT were used to provide more direct evidence for a role of 5-HT in punishment processes.

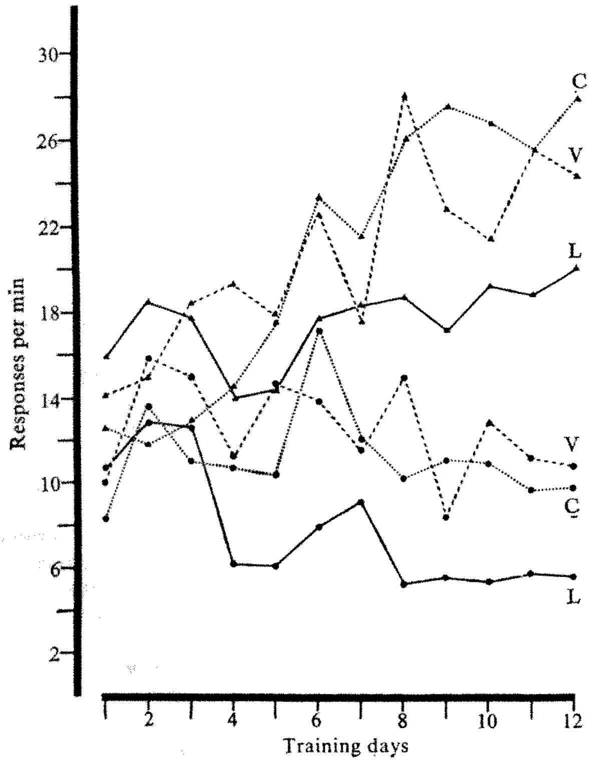
Male Sprague–Dawley rats (250–300 g) were trained to press a lever for food reward on a continuous reinforcement (CRF) schedule. Following this they were randomly divided into three groups. 5,7-DHT creatinine sulphate (Regis) ($n = 8$, 2 μg per 2 μl free base, on each side of brain) or vehicle ($n = 5$, 1 mg ml^{-1} ascorbic acid in sterile isotonic saline) was injected (1 μl min^{-1}) bilaterally into the ventromedial tegmentum. Operated control animals ($n = 5$) underwent the same procedure except that the injection needle was not lowered into the brain. This latter group was included to assess any behavioural disturbances due to 5-HT depletion in vehicle injected animals observed in an earlier pharmacological study. Ether anaesthesia was used and all animals received desmethylimipramine (25 mg per kg body weight, intraperitoneally, since this prevents the effects of 5,7-DHT on NA neurones)[11,12] 45 min before surgery. The incisor bar was set 3.2 mm above the intra-aural plane and coordinates taken from the lambda (+1 mm) skull suture (caudal, 6.5 mm; ventral, 11.3 mm; lateral, 0.5 mm; injection needle introduced at an angle of 47° to the vertical plane).

After surgery, free-feeding weight was reduced to 85% and the effects of the surgical treatments were examined on the acquisition of a multiple three component schedule involving two reward components and a time-out period. The three components were signalled by different light conditions—during the first reward component (10 min) the illumination was provided by the house light alone, time-out (4 min) was signalled by darkness; and during the second reward component (4 min) both the house light and lever lights were on. This sequence, reward (1), time-out, reward (2) was presented twice during each testing session. Reinforcement in the reward components was available on a variable interval (VI) 25 s (with limits of 5 and 55 s) schedule. No reinforcement was programmed during time-out. Animals were tested on this schedule for 12 consecutive days, (training days 1–12).

During the following 6 d (days 13–18) the second reward component was changed to a conflict situation—reinforcement was again available on the same VI schedule. Each reinforced response, however, was also punished (0.8 mA electric shock of 0.5 s to the feet).

Mean responses per minute over successive training days 1–12 for each surgical group in the reward (1) and time out components are shown in Fig. 1. Although lesioned animals tended to respond at a lower rate no overall significant differences were observed between groups during 12 d of acquisition training on the two reward components of the multiple schedule (Kruskal–Wallis, reward (1) $H = 2.62$, $P > 0.20$; reward (2), $H = 4.45$, $P > 0.10$). Furthermore rates of responding in these two components did not differ within each surgical group. But a similar analysis showed a significant overall difference between groups during acquisition of the time-out component ($H = 13.79$, $P < 0.01$). Inspection of the data showed that this was due to less responding by the lesioned animals.

Fig. 1 Response per minute for each surgical group (C, control; V, vehicle; L, lesion) during the training days 1–12 (postoperative days 11–23). ▲, Responding during reward (1); ●, responding during time-out component. The three experimental groups are denoted with different line markings.



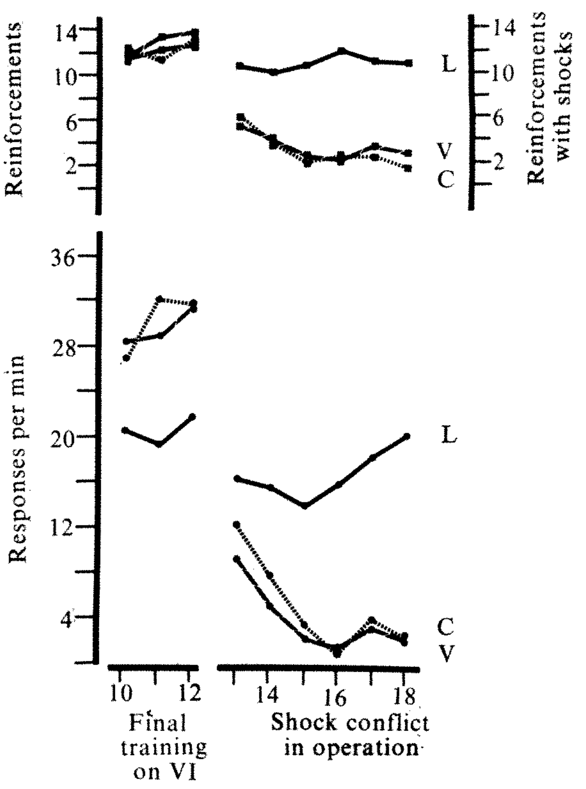


Fig. 2 Third schedule component. Left axis, responses per min and mean reinforcements received for each surgical group (during reward component 2) on the final three training days. Right axis, responses per min (●) and mean reinforcements plus shocks (■) during reward component 2 (now conflict component) for each surgical group (C, control; V, vehicle; L, lesion) on the 6 days of conflict following acquisition training. The experimental groups are denoted with different line markings.

The effects of shock introduction on response rate and shock acceptance in the former reward (2) component are shown in Fig. 2. This manipulation resulted in a dramatic decrease in response rate in both the control and vehicle groups. No suppression was observed in the lesioned animals, however, and correspondingly these animals accepted more shocks. These differences were highly significant overall (responses, $H = 11.56$, $P < 0.01$; shocks, $H = 12.38$, $P < 0.01$) and were observed until the end of behavioural testing on post-operative day 77. No significant overall differences were observed during reward (1) ($H = 3.66$, $P > 0.10$) although lesioned animals continued to respond at a lower rate during time-out ($H = 6.68$, $P < 0.05$).

For biochemical analysis animals were killed on post-operative days 97 and 98 and amine uptake into cortex slices and hypothalamic synaptosomal preparations determined[13,14]. With respect to controls, lesioned animals showed highly significant reductions in 5-HT uptake both in the cortex (77%) (Mann–Whitney test $P < 0.002$) and hypothalamus (66%) ($P < 0.004$) while the vehicle group showed much smaller non-significant reductions (cortex, 25%; hypothalamus, 7.5%). Uptake of noradrenaline in the cerebral cortex was not significantly altered (Mann–Whitney, $P > 0.8$) in any group of animals (vehicle 11%, 5,7-DHT 15% increases of sham). Uptake of hypothalamic noradrenaline was not measured in these experiments but identical 5,7-DHT lesions in a separate series had no significant effect on this parameter, while producing the same pattern and degree of 5-HT denervation[15]. Everitt (in preparation) has also shown no changes in dopamine uptake in septum or striatum after 5,7-DHT lesions in the MFB. Histological analysis of the lesion procedure has shown the canula placement to be localised in the decussation of the brachium conjunctivum dorsal to the interpeduncular nucleus.

Taken together these findings provide strong support for the postulation of a 5-HT 'punishment' system that is critically involved in the acquisition of behavioural suppression in conflict situations. In other experiments, previously acquired suppression was also found to be released by the 5,7-DHT lesion. The punishment-releasing effect of 5-HT depletions does not seem to reflect a nonspecific concommitant of a general release syndrome since the lesioned animals did not show higher rates of responding during the other schedule components. Reduced effectiveness of the electric shock in suppressing responding could be attributed to a reduced pain sensitivity. This is an unlikely interpretation of the results, however, as morphine, a classic analgesic does not re-instate shock-suppressed responding[16] and Hole[17] has reported that 5,7-DHT injections at sites in the midbrain similar to those used here have no effect on pain sensitivity.

It would be interesting to establish if chlordiazepoxide induces characteristic behavioural effects in 5,7-DHT lesioned rats. The most important of these effects is the ability of the minor tranquilisers to reinstate responding suppressed by punishment. But, this is clearly difficult to investigate when, as we have shown, the 5,7-DHT lesion itself releases punished responding. Rates of responding during conflict should be equivalent in all groups of animals before the effects of an anxiolytic drug are evaluated. Preliminary investigations of this question were conducted on the present animals, when the initial conflict experiment and a study of the effects of amphetamine on this behaviour had been completed. On postoperative days 31–34 the animals were restabilised on the basic three-component conflict schedule. During the next 12 d the shock level during the conflict component was increased from 0.8 mA to 2.5 mA in four steps (1.3, 1.6, 2.0, 2.5 mA). The effects of chlordiazepoxide (1.26, 2.0 mg per kg) were studied. The increased shock level decreased significantly conflict responding in the lesioned animals. Chlordiazepoxide did not re-instate responding in these animals to the same degree seen in the control animals. These results may reflect a block of CDP action in the 5,7-DHT-lesioned rats. Caution must be exercised, however, because the lesioned animals were responding in conflict at a higher rate than controls despite the raised shock level. It is therefore possible that under CDP the lesioned rats reach their highest possible rate of responding sooner and thus a response-releasing effect of the normal magnitude was not possible. But it is tempting to speculate that 5-HT pathways have a central role in mediating both the effects of shock on responding and the reversal of these effects with benzodiazepines.

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Intranigral kainic acid is evidence that nigral non-dopaminergic neurones control posture

THE substantia nigra has a key role in the control of posture and in the coordination of movements. This function is currently attributed to the dopaminergic neurones ascending from the pars compacta of the nigra to the neostriatum. In fact unilateral lesion of this pathway by intranigral injection of 6-hydroxydopamine (6OH-DA) produces a dramatic asymmetry consisting of a tendency of the animal to turn towards the side of the lesion (ipsilateral turning)[1]. But, unilateral electrolytic lesions of the substantia nigra, while similarly effective to 6OH-DA in destroying dopaminergic neurones, result in turning towards the intact side (contralateral turning)[2,4]. This has been taken to indicate the existence of non-dopaminergic neurones arising from or traversing the nigra, which control turning behaviour in a manner opposite to the nigro-neostriatal dopaminergic neurones[2,4]. We report here that unilateral intranigral injection of kainic acid[5,6] produces a sustained contralateral turning independent from dopaminergic nigro-striatal function. Since kainic acid spares axons but produces neuronal loss in the pars reticulata of the nigra, our results indicate the existence of non-dopaminergic neurones originating in the pars reticulata which control turning behaviour and posture in a manner opposite to the nigrostriatal dopamine (DA) neurones.

Male Sprague–Dawley rats (290–310 g) were anaesthetised with Equithesin (3.5 mg per kg) and placed in a Stellar Stoelting stereotaxic apparatus; kainic acid (in saline) or 6OH-DA (8 μg of base in saline with 0.1% ascorbic acid) were injected in the substantia nigra using stereotaxic coordinates A-3, L 2, V 7.8, according to the Pellegrino and Cushman[7]. The total injection volume was 0.3 μl, injected at a constant rate of 1 μl per 18 min, through a 0.25-mm stainless-steel cannula. Unless specified, kainic acid was injected in the nigra at the dose of 0.75 μg. Twenty-four hours after intranigral kainic acid the intensity of turning behaviour was assessed as described in the legend to Table 1. Ten days after intranigral kainic acid 42 rats were killed, the brain perfused with formalin, included in paraffin and the mesencephalon cut into serial 20-μm slices which were stained with Luxol fast-cresylviolet in order to ascertain the location of the cannula tip and the extension of the lesion. In another series of rats, injected with kainic acid 10 d previously, the nigra of each side was punched out with stainless-steel cannulas from two 400-μm slices made by cryostat; glutamic acid decarboxylase (GAD), tyrosine hydroxylase (TH) and choline-acetyltransferase (CAT) were assayed according to McGeer and McGeer[6] with slight modifications.

Figure 1 shows the histological appearance of the substantia nigra injected with kainic acid 10 d previously as compared with that injected with saline, in sections stained with cresylviolet–Luxol fast blue. In the lesioned nigra the pars reticulata reveals moderate loss of neuronal perikarya and gliosis, characterised by proliferation of non-neuronal elements. The pars compacta seems less affected than the pars reticulata and still shows the presence of numerous large globose neurones. There are no signs of demyelinisation in or around the substantia nigra.

Intranigral injection of kainic acid produces homolateral turning which appears as soon as the animals recover from the anaesthesia and dominates the acute stage of the symptomatology, lasting about 6–12 h. As the ipsilateral turning subsides rats undergo a variable phase of intermittent ipsi- and contralateral turning but by 24 h after kainic acid administration they show an asymmetric posture with turning contralateral to the lesion.

As shown in Table 1, the intensity and the incidence of the contralateral turning is related to the dose of kainic acid, being maximal after 1.0 μg.

The contralateral turning is maximal during the first 3–4 d

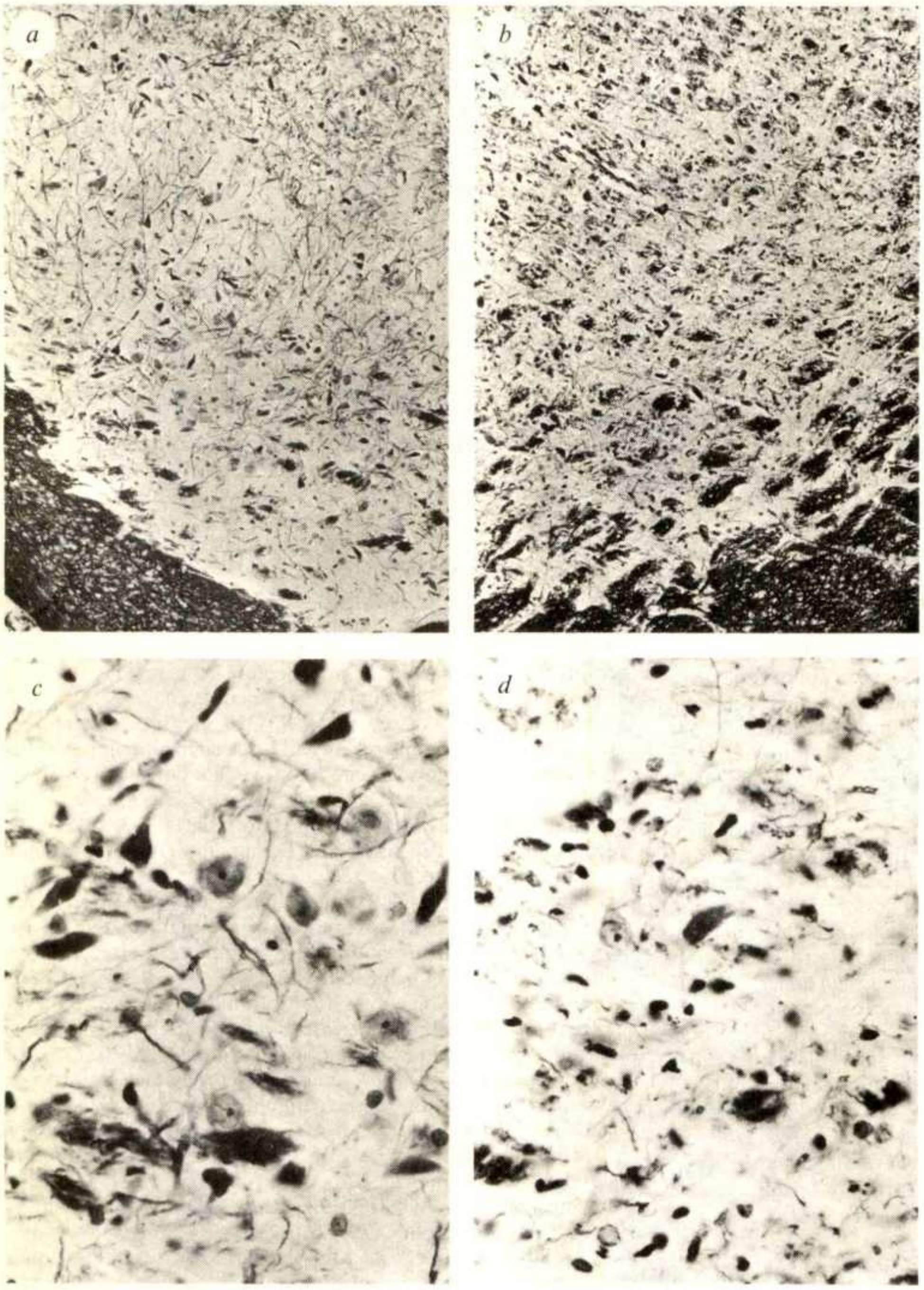

Fig. 1 Micrographs of Luxol fast–cresylviolet stained sections of substantia nigra pars reticulata from a rat injected unilaterally in the nigra with 0.75 µg of kainic acid, 10 d previously. *a*, *c*, Control side; *b*, *d*, kainic-injected side. *a*, And *b*, ×5; *c* and *d*, ×20.

after kainic administration and, although diminished in intensity, is still present 10 d later. Failure to obtain the chronic contralateral turning was associated, in 10 out of 12 rats, with placement of the cannual tip outside the substantia nigra. Among 30 rats where contralateral turning was obtained, the tip of the cannula was found to be placed in the centre of the pars reticulata in 16, at the border between the compacta and the reticulata in 8, in the pars lateralis in 4 and in the medial part of the nigra in 2 cases. Ten days after intranigral kainic acid nigral GAD and CAT in the kainic-injected side are not significantly different from the contralateral one while nigral TH is decreased by about 30% in the kainic-injected side (GAD: 965±45 compared with 987±52; CAT: 8.8±0.8 compared with 8.3±0.6; TH: 11.8±0.6 compared with 8.2±0.5; means ± s.e.m. of 10 determinations in control compared with kainic-injected side expressed in nmol per h per mg protein).

Bilateral intranigral administration of 6OH-DA, which decreased nigral TH-activity by more than 85% in respect to controls (see legend to Table 1), failed to affect the induction of chronic contralateral turning by kainic acid injected unilaterally in the substantia nigra (see Table 1).

Our results show that unilateral intranigral administration of kainic acid produces chronic contralateral turning. This effect was independent from an action on nigro–neostriatal DA-neurones, as it took place also when they had been previously destroyed bilaterally by intranigral 6OH-DA. Kainic acid produced a distinct loss of nigral neurones but did not damage myelinated fibres or change the activity of nigral GAD, a marker of afferent GABA-connections[8-10]. This indicates that the contralateral turning produced by intranigral kainic acid is due to destruction of nigral neurones rather than to damage of fibre tracts afferent to the nigra or passing near it.

Since the chronic effects of the kainic-induced nigral lesion are just opposite to the selective destruction of the nigrostriatal DA-neurones with 6OH-DA and are in turn unrelated to their intactness, our results indicate the presence, in the substantia nigra, of neurones controlling turning behaviour and posture in a manner opposite to the dopaminergic ones. Stimulation of

Table 1 Incidence and intensity of controlateral turning after various doses of intranigral kainic acid

Kainic acid (μg)	6OH-DA (8 μg)	Rats injected	Rats turning with score				Turns per 5 min*
			0	1	2	3	
0.25	—	10	8	2			—
0.50	—	15	5	2	7	1	15
0.75	—	30	6	2	6	16	32±5
0.75	+	20	3	1	4	12	35±7
1.0	—	20	1	1	4	14	38±6

Turning was scored 24 h after unilateral intranigral kainic acid according to Costall *et al.*[4]. 6OH-DA was administered in the nigra of both sides 15 d before kainic acid. In these rats, nigral TH was decreased by about 87% in respect to controls (1.5±0.4 compared to 12.2±2.5, mean ± s.e.m. of five determinations).

*Values are mean ± s.e.m.

these non-DA neurones by kainic acid would explain the homolateral turning obtained in the acute stage after intranigral kainic acid.

The chronic effects of the intranigral administration of kainic acid are strikingly similar to those obtained by electrocoagulative lesions of the nigra[2–4]. This suggests that the effects of the electrolytic lesions are due to destruction of nigral neurones rather to damage of fibre tracts.

As to the nature of the neurones destroyed by kainic acid, the fact that the loss of nigral perikarya is confined to the pars reticulata and that CAT activity is within the normal range, suggests that the lesion produced by kainic acid is limited to a fairly specific neuronal population. Indeed this would not be unexpected since the toxic effect of kainic acid, being related to stimulation of specific glutamate receptors[11], should vary among different neuronal types depending on the density of glutamate receptors on their perikarya. Accordingly, the relative intactness of the pars compacta and the minor reduction of nigral TH after intranigral kainic acid as compared with the dramatic decrease of striatal GAD and CAT after intrastriatal kainic acid[5,6], suggests that the dopaminergic nigral perikarya are much less sensitive to the toxin than striatal GABA-ergic and cholinergic perikarya.

The histological procedure adopted in this study does not permit the morphological identification of the nigral neurones destroyed by kainic acid. But, in view of the existence of nigral non-DA neurones projecting to the thalamus, to the reticular formation or to the striatum[12–16], lesion of these pathways could be the cause of the chronic effects of intranigral kainic acid.

The existence of nigral non-DA neurones controlling turning behaviour in a manner opposite to the nigrostriatal DA-system can provide an explanation for some turning responses obtained from the nigra which are not readily explained by an action on the nigrostriatal DA-system. For example, unilateral intranigral administration of ethanolamine-*O*-sulphate, which produces an accumulation of endogenous GABA by blocking GABA-transaminase, results in spontaneous contralateral turning[17,18]. This syndrome resembles the chronic one produced by intranigral kainic acid and therefore might be due to a GABA-mediated depression of those nigral neurones destroyed by kainic acid.

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Enkephalin receptors on dopaminergic neurones in rat striatum

THE idea that morphine and other narcotic analgesics as well as opioid peptides affect the activity of nigrostriatal dopaminergic neurones is well supported. For instance these substances elicit changes in motor activity similar to those observed after blockade of dopamine (DA) receptors by neuroleptics, they increase DA turnover in striatum and several typical symptoms of the abstinence syndrome they provoke involve dopaminergic systems (see refs 1–3 for reviews). In agreement with these observations, a high density of 'opiate receptors'[4] and of enkephalin binding sites[5,6], a high content in enkephalins[7] and a dense network of enkephalin-containing neurones[8] are found in the striatum. All these observations suggested the possible connection between alleged enkephalinergic neurones and the dopaminergic fibres ending in the striatum. We report here that both the regional distribution of high affinity (morphine-displaceable) binding sites for leucine-enkephalin (Leu-enkephalin) in striatum and their decreased number after chemical or mechanical interruption of the nigrostriatal bundle support the assumption that enkephalinergic neurones terminate presynaptically on dopaminergic nerve terminals in the striatum.

^{3}H-Leu-enkephalin binds to a particulate fraction from rat striatum and the saturation kinetics at equilibrium evidence two distinct sites; the first one, exhibiting a high affinity (K_d around 3 nM) and recognised by morphine or naloxone, has been identified as the 'opiate receptor' whereas the nature and possible function of the second one is not yet established[5,6]. We have determined the number of binding sites for ^{3}H-Leu-enkephalin displaceable by morphine in successive frontal sections of rat striatum (Fig. 1). The density of these sites decreases regularly from the head to the tail of the striatum, with a twofold variation between the first and the last dissected area. The topographical variation of DA content (Fig. 1), in agreement with the report of Tassin *et al.*[11], grossly parallels that of the sites of ^{3}H-Leu-enkephalin binding displaceable by morphine. Since

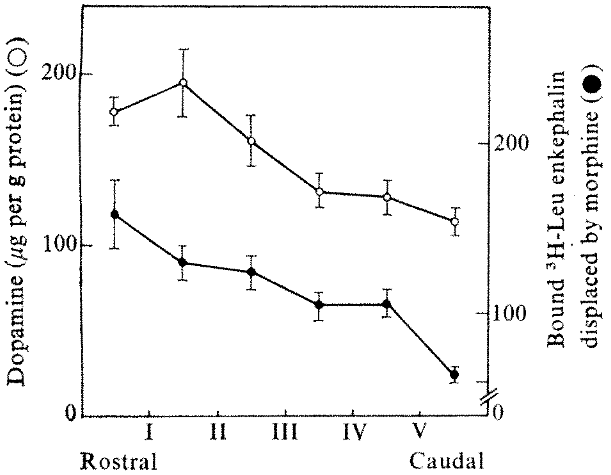

Fig. 1 Distribution of ^{3}H-Leu-enkephalin binding sites displaceable by morphine and DA content in frontal sections of rat striatum. The rat striatum was dissected according to Glowinski and Iversen[9], applied on a cold plate and divided into six serial slices. The first section was performed at the level of the anterior commissure (arrow) and therefore slice I included a portion of n. accumbens in addition to striatal tissue; the remaining tissue was then divided into five parallel slices of equal thickness. The good reproducibility of the dissection procedure was indicated by a variation of less than 6% between the protein content of homologous slices in different experiments. Homologous slices from four animals were pooled together and homogenised in 20 volumes of cold 50 mM Tris buffer, pH 7.4. An aliquot of the homogenate was quickly removed and deproteinised with $HClO_4$ for DA assay[10]. Binding of ^{3}H-Leu-enkephalin[5,6] was measured on a particulate fraction of the homogenate obtained by two successive centrifugations (180*g* for 5 min, then 10,000*g* for 20 min). 15-min incubations at 30 °C in the presence of 20 μM bacitracin, and 12 nM ^{3}H-Leu-enkephalin (45.6 Ci mmol^{-1}, Radiochemical Centre, Amersham) alone or in the presence of 5 μM morphine. Bound ^{3}H-Leu-enkephalin is expressed as fmol per mg of protein and represents the binding displaceable by morphine. Means ± s.e.m. from five separate experiments with triplicate assays for ^{3}H-Leu-enkephalin binding and duplicate DA estimations. ○ Dopamine content. ●, ^{3}H-Leu-enkephalin binding sites.

the variation in DA content reflects the heterogeneous distribution of DA terminals, our results suggest that enkephalin receptors might be topographically associated with dopaminergic synapses.

The presence of enkephalin receptors on DA neurones themselves was investigated by evaluating the effects of selective lesions of the nigrostriatal neurones on ^{3}H-Leu-enkephalin binding. Degeneration of DA terminals was elicited either chemically by 6-hydroxydopamine (6-OHDA) or by electrocoagulation of the pars compacta of substantia nigra and neurochemically assessed by the reduction in 'dopa' decarboxylase activity (Table 1). Such lesions resulted after 6–22 d in significant decreases in ^{3}H-Leu-enkephalin binding displaceable by morphine. The similar decrease in ^{3}H-naloxone binding following the chemical lesions confirms that the high affinity binding sites of ^{3}H-Leu-enkephalin represent the 'opiate receptors'. To confirm that the observed changes were indeed related to the degeneration of DA neurones, we have more selectively destroyed the nigrostriatal DA system by careful unilateral microinjections of 6-OHDA into the substantia nigra in the conditions of Agid *et al.*[12]. Two weeks later the decrease in ^{3}H-Leu-enkephalin binding displaceable by morphine observed in the striatum of the lesioned side was by 27 ± 3% (means ± s.e.m. from nine animals) whereas the high affinity uptakes of ^{3}H-choline and ^{3}H-5-hydroxytryptamine[13] in striatal synaptosomes from the same animals were not significantly modified. In addition, we have observed significant decreases in ^{3}H-Leu-enkephalin and ^{3}H-naloxone binding (by 30–40%) in rats with the nigrostriatal bundle sectioned at the hypothalamic level 10–30 d before (in preparation).

The decrease in ^{3}H-Leu-enkephalin binding following electrocoagulation of the substantia nigra probably reflects a diminished number of receptor sites since the decrease affects the maximal binding on the high affinity sites without significant change in K_d (4.7 ± 0.8 nM on the control side compared with 4.2 ± 0.4 nM on the lesioned side) (Fig. 2).

Conceivably transynaptic degeneration may have a role in the observed changes but our observation, consistent with previous reports[14,15], that ^{3}H-choline uptake is not affected indicates that striatal cholinergic neurones, on which DA neurones project, do not degenerate transynaptically. It might also be argued that the decreased number of Leu-enkephalin binding sites is due to the interruption of a neuronal system other than the dopaminergic one (or the serotoninergic one which is unaffected after intranigral 6-OHDA). Such an hypothesis cannot be entirely ruled out but seems very unlikely because the decreased binding is elicited by three different types of lesions—electrolytic, mechanical and chemical—of which the common feature is to induce a degeneration of the DA terminals in the striatum.

Hence both the regional distribution of the high affinity Leu-enkephalin binding sites within the striatum and the decreased number of these sites after lesions might be interpreted on the assumption that enkephalinergic (opiate) receptors are localised on dopaminergic terminals. Assuming that the high affinity Leu-enkephalin binding sites represent postsynaptic receptors for the alleged enkephalinergic neurones, our findings would suggest that these neurones project presynaptically on to dopaminergic nerve terminals. This idea is strengthened by the immunohistochemical demonstration of Leu-enkephalin containing nerve

Table 1 Effects of substantia nigra lesions and intraventricular injections of 6-OHDA on binding of ^{3}H-Leu-enkephalin or ^{3}H-naloxone in rat striatum

Lesions	^{3}H-Leu-enkephalin binding displaceable by morphine (fmol per mg protein)	^{3}H-Naloxone binding (fmol per mg protein)	Dopa decarboxylase activity (nmol per mg protein per h)
Chemical lesion by intraventricular 6-OHDA			
Control animals	162 ± 10	66 ± 3	0.31 ± 0.02
Lesioned animals	126 ± 11*	47 ± 2‡	0.20 ± 0.02†
Variation	−22%	−28%	−33%
Electrocoagulation of the substantia nigra			
Control side	175 ± 11		0.30 ± 0.02
Lesioned side	134 ± 8*		0.19 ± 0.02‡
Variation	−23 ± 2%*		−37 ± 4%

For the chemical lesions the rats received intraventricularly 250 μg of 6-OHDA dissolved in 10 μl of saline containing 2% ascorbic acid. Controls received the same volume of vehicle. Unilateral electrocoagulations of the substantia nigra, pars compacta, were performed under chloral anaesthesia by passing a current of 80 mA for 5 s. Stereotaxic coordinates ($A = +1.8$ mm, $L = 1.8$ mm, $V = -2.3$ mm) refer to the König and Klippel atlas. Animals were killed 6–22 d after placement of lesions. Placement of electrocoagulations was checked histologically. ^{3}H-Leu-enkephalin binding displaceable by morphine was estimated as described in Fig. 1 and typically represented about 1,000 d.p.m. per sample (0.15 mg protein) in controls; triplicate assays varied by less than 5%. Saturable ^{3}H-naloxone binding was estimated on aliquots of the same particular fraction incubated with 3 nM ^{3}H-naloxone (20 Ci mmol^{-1}, NEN Chemicals) alone or in the presence of 1 μM non-radioactive naloxone. DOPA decarboxylase activity was measured, on aliquots of the initial homogenate incubated for 40 min in the presence of 8×10^{-4} M ^{14}C-carboxyl-labelled Dopa (Radiochemical Centre, Amersham). Means ± s.e.m. of five to eight separate experiments, on pools from one to three animals each.

*$P < 0.05$
†$P < 0.01$
‡$P < 0.005$.

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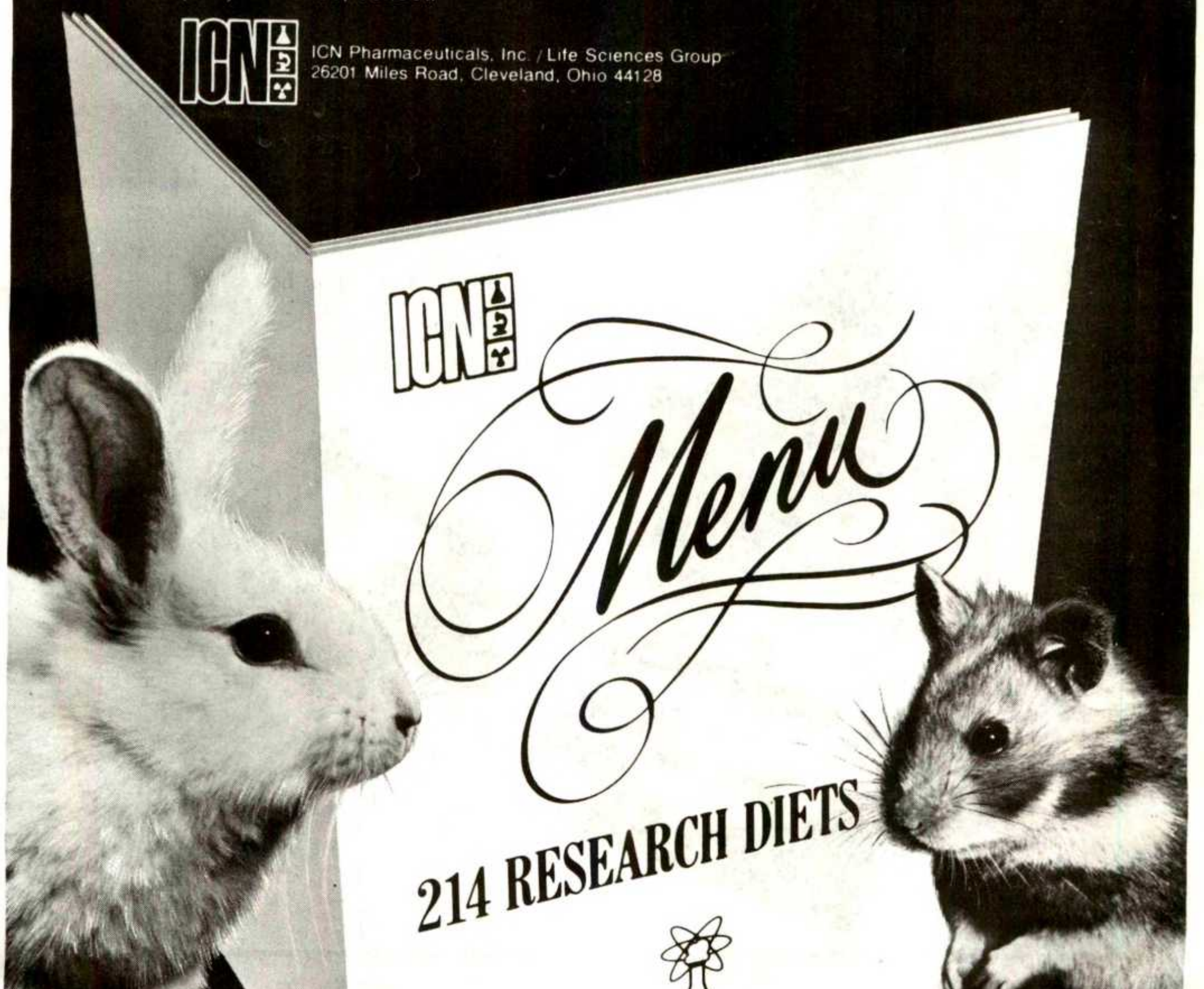

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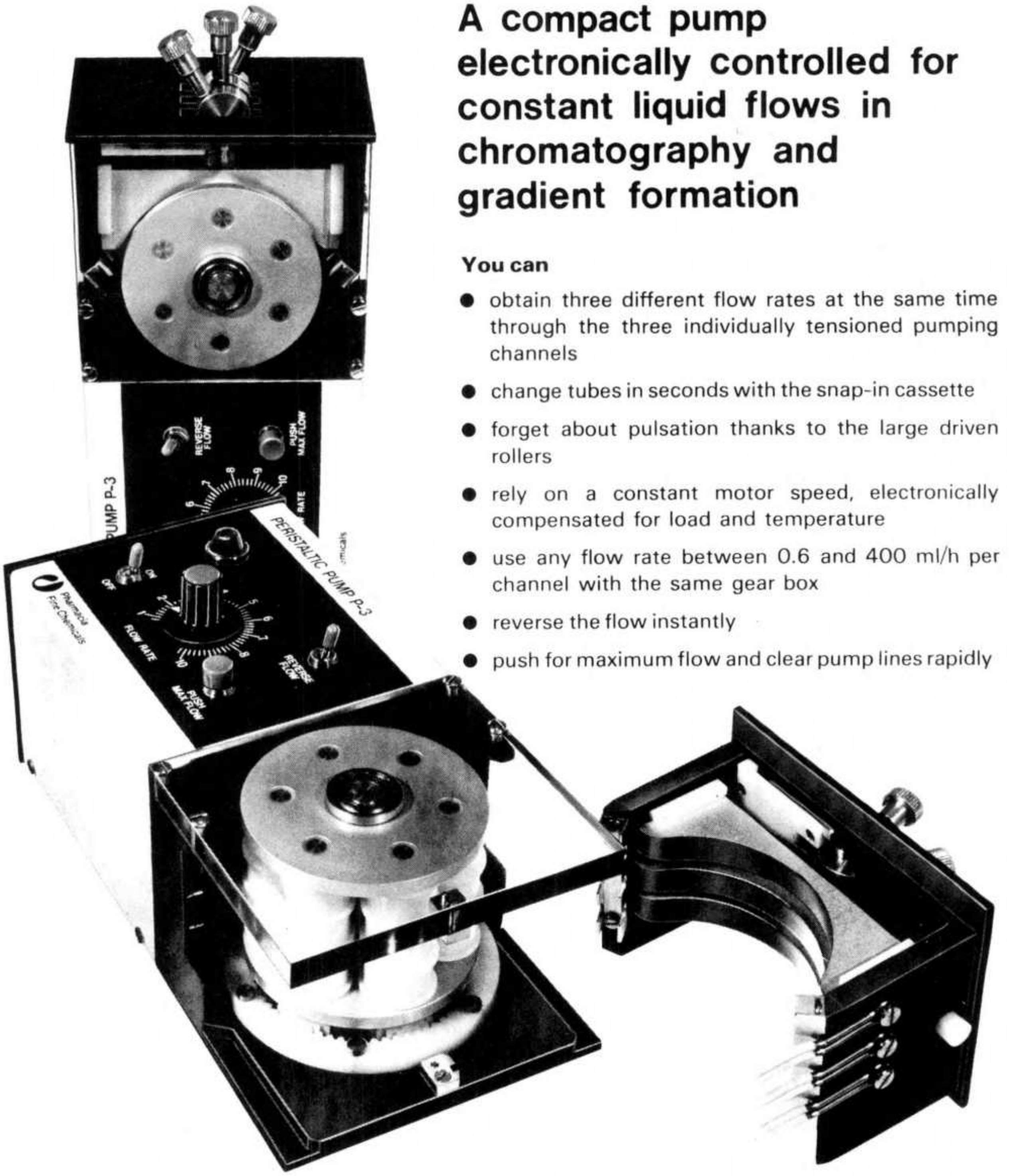

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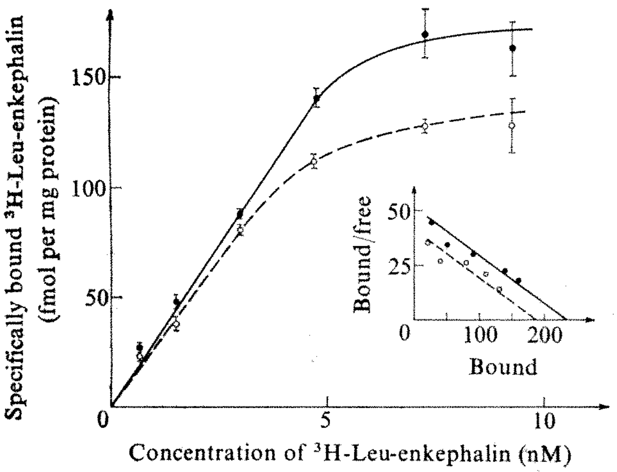

Fig. 2 High affinity binding of ^{3}H-Leu-enkephalin to striatal membranes after unilateral electrocoagulation of the substantia nigra, pars compacta. Rats killed 10 d after placement of the lesions, as described in Table 1. Specifically bound ^{3}H-Leu-enkephalin refers to the binding displaceable by 50 µM non-radioactive Leu-enkephalin. Means ± s.e.m. from triplicate determinations on pooled striata from four animals. Insert: Scatchard plots of the same data. ●, Control side; ○, lesioned side.

terminals in rat striatum[8], by the presence of the peptide in synaptosomal fractions from this region[7,16] and finally, by the effect of morphine and opioid peptides on the release of DA from striatal slices[17,18].

The limited decreases in Leu-enkephalin receptors following our lesions indicate that only a fraction of these receptors in striatum are present on DA neurones. Even after sections of the nigrostriatal bundle at the hypothalamic level, resulting in an extensive depletion (>80%) of striatal DA the decrease in ^{3}H-Leu-enkephalin binding was only 30–40%. This suggests that about one-third of enkephalin receptors in striatum are present on dopaminergic nerve terminals and that non-dopaminergic cells in the striatum might also receive enkephalinergic inputs.

From observations that morphine and endorphins decelerate the K$^+$-induced release of DA slices of rat striatum[17,18], it might be inferred that the effect of enkephalin receptors on dopaminergic neurones could be to modulate DA release in an inhibitory fashion. This would account for the symptoms of a decreased dopaminergic neurotransmission, such as catalepsy, following the administration of morphine or endorphins to rats[19]; the paradoxical increase in DA synthesis might be only a secondary effect like that observed after various treatments resulting in a diminished release of the catecholamine[20,21].

There is already evidence that opiate receptors are also localised presynaptically on sensory fibres of the vagus nerve and the spinal cord[22]. In addition morphine and endorphins reduce the release not only of DA but also of other cerebral neurotransmitters like acetylcholine or noradrenaline[3]. Taken together with our data, these observations suggest that a major function of enkephalins could be to mediate presynaptic inhibitions on a variety of neuronal systems in the central nervous system.

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Blowfly ovipositor receptor neurone sensitive to monovalent cation concentration

THE ability of insects to taste salts with sense organs on their legs and mouthparts is well documented. Studies of taste hairs on the tarsi and labella of the black blowfly *Phormia regina* and the bluebottle *Calliphora erythrocephala* have shown that these contain several sensory cells, one of which is a 'salt' or 'type I' primary receptor neurone responding positively to the concentration of monovalent cations[1–6]. I report here some of the receptor characteristics of monovalent cation sensitive neurones found in hair sensilla on the egg-laying apparatus of the sheep blowfly.

Sensory hairs with terminal pores have been identified on the ovipositors of the Australian sheep blowfly *Lucilia cuprina* and the migratory locust *Locusta migratoria*[7,8]. As there is much behavioural evidence to suggest that insects taste their oviposition medium with their ovipositors[9–12], it is likely that the newly described sensilla are the gustatory receptors that mediate such behavioural reactions. In *L. cuprina* these sensilla have been shown, by electrophysiological recording, to contain at least three neurones which respond to stimulation with various chemical solutions[7]. Some gustatory sensilla of caterpillars respond to olfactory stimuli[13], however, and as the ovipositor of the blowfly *P. regina* is known to make directed movements in response to olfactory stimuli[14], it is of interest to know more about the receptor properties of blowfly ovipositor sensilla.

Gravid female *L. cuprina* from our culture of the GL strain were used. Conventional stimulating and recording equipment was used following the procedure of Hodgson *et al.*[15]. The glass micropipette containing the stimulating solution and recording electrode was placed over the tips of each of the 1g–5g sensilla[7] in turn. All of the g sensilla were found to contain a neurone whose rate of firing increased with increasing concentration of applied sodium chloride solutions (Fig. 1 shows a typical result).

Sensillum 1g was the easiest to record from and all results given here were obtained from 1g sensilla. During this work recordings have been made from more than eighty 1g sensilla. The oscilloscope time-base was triggered by the contact artefact, a dual sweep displaying one trace at 10 times the speed of the other, the high speed trace sampling the initial part of the slower trace. This enabled analysis both of the initial rate of firing and also of the adaptation rate. The fast trace was also useful for inspection of the action potential shapes (Figs 1, 2).

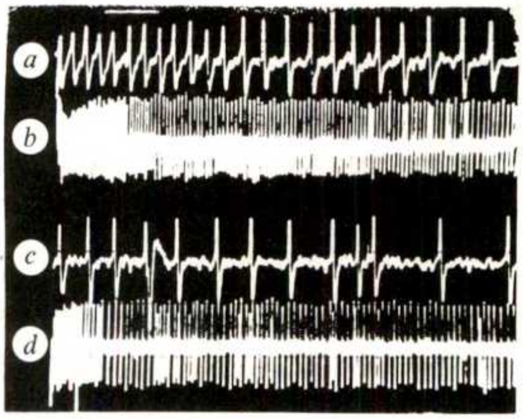


Fig. 1 Effect of NaCl concentration on the initial discharge frequency of the monovalent cation receptor neurone in the 1g sensillum on the ovipositor of *Lucilia cuprina*. *a*, *b*, 1.5 M NaCl; *c*, *d*, 0.15 M NaCl. Traces start at moment of stimulus application. Scale line: 20 ms in (*a*) and (*c*); 0.2 s in (*b*) and (*d*).

Action potentials of three different amplitudes were recorded from the g sensilla on stimulation with salt solutions: small- (1), medium- (2), and large- (3) sized action potentials (Fig. 2). When stimulated with monovalent salts, the labeller taste hairs of *Calliphora erythrocephala* also produce three sizes of impulses which have been tentatively identified as cation, anion and water receptors respectively[16]. In the 1g receptors of *L. cuprina* action potentials from cell 2 are always obtained in response to stimulation with solutions of monovalent cation chlorides, the smaller cell 1 action potentials sometimes accompany cell 2 responses, generally starting to discharge after prolonged stimulation, while the larger cell 3 action potentials are a more rare accompaniment. Responsiveness of the g sensilla varied from fly to fly being influenced by the age and nutritional status of each individual. By comparing the relative stimulating power of a series of cations and anions on single 1g sensilla, replicable results were achieved. Receptor cell 2 was found to be almost equally responsive to 0.15 M solutions of NaCl, NaBr and NaI but much less responsive to solutions of NaF (Fig. 3). The rate of firing of this neurone is largely dependent on the concentration of monovalent cation present in the stimulating solution and, apart from NaF, is not much effected by the anions tested, which included NO$_3^-$ and SO$_4^{2-}$. A range of monovalent cation chlorides were almost equally stimulatory, though the Li$^+$ response had a faster adaptation rate than either Na$^+$ or K$^+$ (Fig. 3).

In contrast to its concentration-dependent responses to monovalent cations, neurone 2 is much less responsive to the divalent cations Ca^{2+}, Mg^{2+} and Sr^{2+} and is totally unresponsive to a range of concentrations of choline chloride. Divalent cations at a concentration of 0.15 M were sometimes stimulatory (Fig. 4) but a 10-fold increase in concentration did not increase the response as it always does with monovalent cations. More details of the responses of 1g sensilla to divalent cations will be published elsewhere.

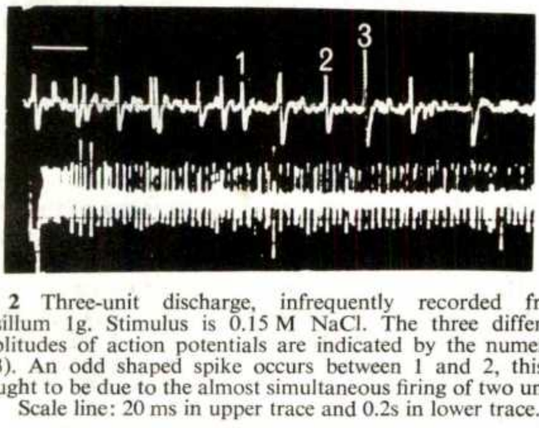
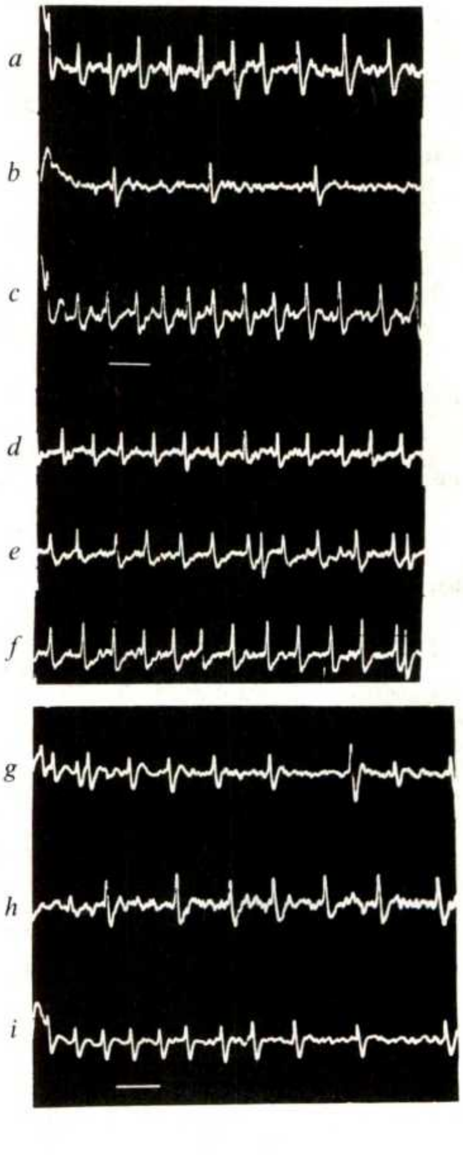


Fig. 2 Three-unit discharge, infrequently recorded from sensillum 1g. Stimulus is 0.15 M NaCl. The three different amplitudes of action potentials are indicated by the numerals (1–3). An odd shaped spike occurs between 1 and 2, this is thought to be due to the almost simultaneous firing of two units. Scale line: 20 ms in upper trace and 0.2s in lower trace.

When ranges of NaCl and KCl solutions were tested a logarithmic relationship was found, with the type 2 cell approximately doubling its initial rate of firing for each 10-fold increase in molar concentration of Na$^+$ or K$^+$ ions. Threshold for cell 2 was as low as 0.001 M NaCl in several preparations, which is 100 times lower than was found in the salt receptors of the labellar hairs of *P. regina*[17]. This may reflect the need of the sheep blowfly to identify this salt with its ovipositor receptors when attempting to lay eggs on diluted serous exudates in the wet wool of sheep, sodium being the main monovalent cation of serum.

The cell 2 receptor neurone in the 1g sensillum of the ovipositor of *L. cuprina* is specialised for detection of monovalent cation concentrations. This neurone is very similar to the 'salt' or 'type I' receptor neurones that are so well known from the taste hairs of the blowfly labellum and

Fig. 3 Single-unit responses from two 1g sensilla in response to 0.15 M salt solutions. Traces *a*–*f*: responses to sodium halides; chloride acting as the control; traces *g*–*i*: responses to monovalent cation chlorides. *a*, NaCl; *b*, NaF; *c*, NaCl; *d*, NaBr; *e*, NaI; *f*, NaCl; *g*, NaCl; *h*, KCl; *i*, LiCl. Scale lines, 20 ms.



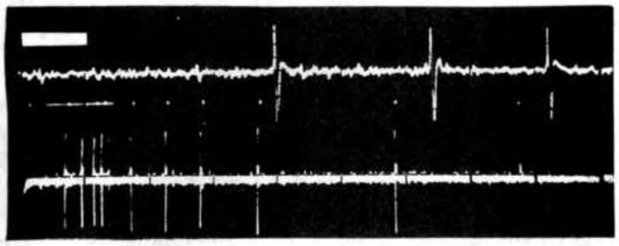


Fig. 4 Single-unit responses from a 1g sensillum to 0.15 M $CaCl_2$ solution. Scale line: 20 ms upper, 0.2 s lower trace.

tarsus[1-6]. This work now adds the ovipositor as the third position in which such receptor neurones are found. The behavioural role of monovalent cation receptors on the sheep blowfly ovipositor is likely to be in the selection of suitable egg-laying sites. When blowflies lay eggs on sheep or on artificial media, probing of the substrate with the ovipositor precedes the deposition of eggs. Locusts and parasitic wasps only lay eggs after the ionic composition of the substrate has been monitored by probing with the ovipositor[9,11]. It is interesting to speculate that alteration of the ion composition on the integument of sheep may influence the egg-laying behaviour of sheep blowflies.

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Non-cholinergic excitatory transmission to intestinal smooth muscle cells

CONTRACTILE responses of the chicken rectum to stimulation of Remak's nerve and intramural nerves are virtually unaffected by drugs affecting the cholinergic or adrenergic system, suggesting that the remaining response is mediated through excitation of nerves other than cholinergic or adrenergic nerves[1-3]. Using the sucrose-gap method we found excitory junction potentials assumed to be elicited by stimulation of excitatory nerves whose transmitter was unknown[4]. In an attempt to confirm and extend these observations we have studied an electrical change in the smooth muscle membrane, recorded intracellularly, in junctional transmission from excitatory nerves.

The rectum was isolated together with Remak's nerve running parallel to the longitudinal axis of the organ and with its several branches extending to the intestinal wall. This was sectioned lengthwise and mounted in a chamber provided with electrodes which allow Remak's nerve and intramural nerves to be stimulated separately. The membrane potential of smooth muscle cells was measured using conventional glass microelectrodes filled with 3 M KCl and having a resistance of 60–100 MΩ.

Stimulation of the anal cut end of Remak's nerve with single square pulses (0.5 ms duration, at a supramaximal intensity) gave action potentials in cells along the entire surface of the rectum in most preparations. In the majority of cells, action potentials arose from slow depolarisations of the membrane potential which range up to 15 mV from the resting membrane potential (-48.6 ± 2.6 mV, $n=80$) and are interpreted to be excitatory junction potentials (EJPs) elicited by a transmitter released from nerve endings (Fig. 1*a*). In some cells, they fired during the falling phase of EJPs or without any preceding changes in the membrane potential.

The amplitude of the EJPs varied both from preparation to preparation and from cell to cell in any one preparation. Their amplitude also varied with the stimulus intensity. It was possible to evoke subthreshold EJPs by decreasing the stimulus voltage. At any stimulus intensity, stimulation of the oral cut end of Remak's nerve was much less effective in producing the electrical response. These results suggest that the smooth muscle cells in the rectum receive a multiple innervation from the excitatory nerves in varied densities and that the excitatory nerve fibres originate mainly in the ascending fibres of Remak's nerve. In all preparations in which cells giving only an EJP were observed, a train of pulses at frequencies less than 10 Hz facilitated EJPs until the depolarisation reached a level to initiate the action potential (Fig. 1*b*). The observed latency before onset of EJPs was 40–65 ms with a mean of 55.0 ± 2.9 ms (±s.e.). Latency of EJPs was not consistent in cells along the longitudinal axis of the preparation, but it did not significantly vary along the transverse axis; as the microelectrode was moved farther away from the stimulating site, the latency prolonged. The increased latency was up to 20 ms in three preparations after shifting the recording site 20 mm away. EJPs took from 55–100 ms to attain the maximum height after the onset and lasted 600–850 ms.

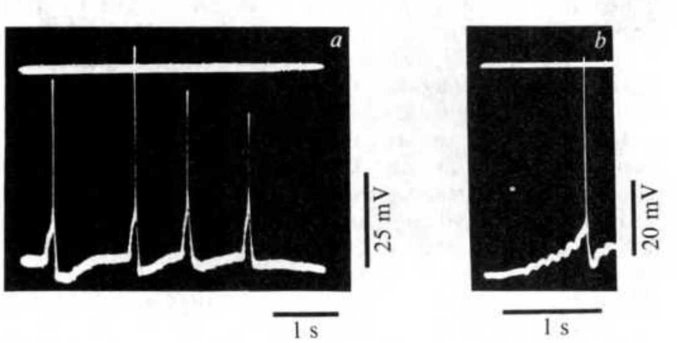

Fig. 1 Intracellular records from smooth muscle cells in the chicken rectum during stimulation with pulses of 0.5 ms duration of Remak's nerve. *a*, Action potentials arose from slow depolarisations of the membrane potential (interpreted to be excitatory junction potentials); *b*, summation of excitatory junction potentials, evoked by repetitive stimulation at 10 Hz, over threshold for initiation of the action potential in another cell.

Field stimulation of intramural nerves (0.1 ms duration) also gave an EJP with latency shorter than those of EJPs elicited by Remak's nerve stimulation. The latencies estimated in EJPs recorded from 30 cells in 8 different preparations varied from 5 to 15 ms with a mean of 9.2 ± 1.2 ms (±s.e.). EJPs in response to Remak's nerve stimulation, but not those to field stimulation, were blocked by hexamethonium (10^{-4} g ml^{-1} or less). Thus, it was apparent that there was ganglionic transmission mediated via acetylcholine receptors of the nicotinic type in the motor pathway from Remak's nerve to the rectum and that the delay of EJPs after the nerve stimulation is largely due to synaptic transmission at the ganglion. Neither atropine nor hyoscience in

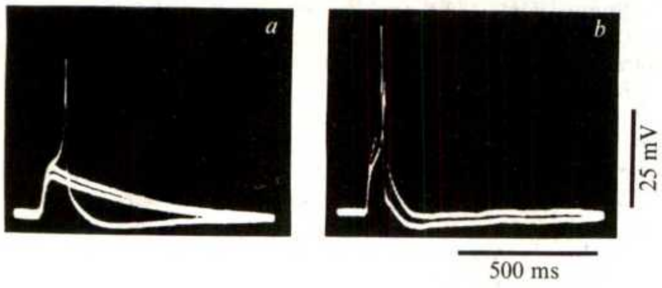

Fig. 2 Effect of atropine on excitatory junction potentials evoked by field stimulation with pulses of 0.1 ms duration at 0.8 Hz. *a*, Control; *b*, 5 min after addition of atropine, 5×10^{-7} l^{-1}, to the bathing solution. Note that the excitatory junction potentials were appreciably potentiated by atropine.

concentrations up to 10^{-6} g ml^{-1} inhibited EJPs evoked by stimulation of the extrinsic and intrinsic nerves and instead these drugs invariably potentiated the EJPs. This effect is illustrated in Fig. 2, where the EJPs are evoked by field stimulation with single pulses at an interval of 1,250 ms before and after application of atropine (5×10^{-7} g ml^{-1}). In these stimulus conditions, facilitation of EJPs was seen in this cell and the third EJP reached the threshold membrane potential to trigger the action potential. In contrast, depolarisation of the first EJP was sufficient for firing the action potential in the presence of atropine. Physostigmine at between 10^{-9} and 10^{-7} g ml^{-1} did reduce the EJPs without change in the membrane potential. Drugs affecting adrenergic functions had no effect on the EJPs. Tetrodotoxin 10^{-7} g ml^{-1} abolished the electrical responses to field stimulation as well as Remak's nerve stimulation.

It is very likely that neurones directly involved in generation of the EJPs are neither cholinergic nor adrenergic. It is possible that there is a muscarinic mechanism by which the motor transmission to the smooth muscle cells is inhibited.

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Calcium transients in frog slow muscle fibres

MANY muscles in the frog contain slow as well as twitch muscle fibres, which differ in their innervation, electrical and contractile properties and fine structure[1]. Slow fibres give graded slow contractions with nerve stimulation, and can maintain a prolonged contracture when depolarised. There is evidence that contractile activation in slow fibres is mediated by a rise in myoplasmic calcium concentration[2], but it is not clear whether this calcium originates from the sarcoplasmic reticulum, as in twitch fibres, or enters the fibre from the external solution[1,3–5]. We have used the calcium indicator dye arsenazo III (refs 6–9) to follow changes in intracellular free calcium concentration occurring during depolarisation of slow fibres, and find that the membrane potential dependence of these calcium transients in slow fibres is very similar to that observed in twitch fibres[9]. The time courses of the calcium transients in slow fibres are, however, very much slower than in twitch fibres[9,10], and may be a major factor in determining the time courses of tension development and relaxation.

Experiments were performed on the pyriformis muscle of *Rana temporaria*[11], using a bathing solution of composition (mM); NaCl, 120; KCl, 2; $CaCl_2$, 12; *p*H, 7. Intracellular calcium changes with arsenazo III were measured as previously described for twitch fibres[8,9]. Briefly, by cutting away the lower part, the muscle was reduced to a thin sheet of fibres which was stretched until contraction was practically abolished. Fibres were impaled with two electrodes, about 150 μm apart, for voltage recording and dye injection. Slow fibres were identified by their inability to generate action potentials and their slow electronic time constant when tested with current pulses applied through the dye pipette. The membrane potential was held at −100 mV by passing a hyperpolarising current through the dye pipette, and this served also to slowly inject arsenazo III into the fibre. Changes in light absorption of the dye were monitored by focusing light from a 50-W bulb on to a spot of about 80 μm diameter between the pipettes, and measuring the transmitted light at wavelengths of 532 and 602 nm using two photomultipliers and interference filters. The light absorbance of arsenazo III changes in opposite directions at these wavelengths in the presence of calcium, and by subtracting the signals from the two photomultipliers a record is obtained which gives a quantitative measure of changes in free calcium concentration, but is little affected by movement artefacts[8,9].

Changes in intracellular free calcium concentration produced by depolarising pulses are shown in Fig. 1. The size of the calcium transient is a graded function of membrane potential, and the rates of increase and decay of calcium concentration are much slower than in twitch fibres[9,10]. The decay time constant measured in six fibres varied between 0.6 and 4 s, and the maximum rate of increase in free calcium concentration in 10 fibres was 0.045 μM ms^{-1}

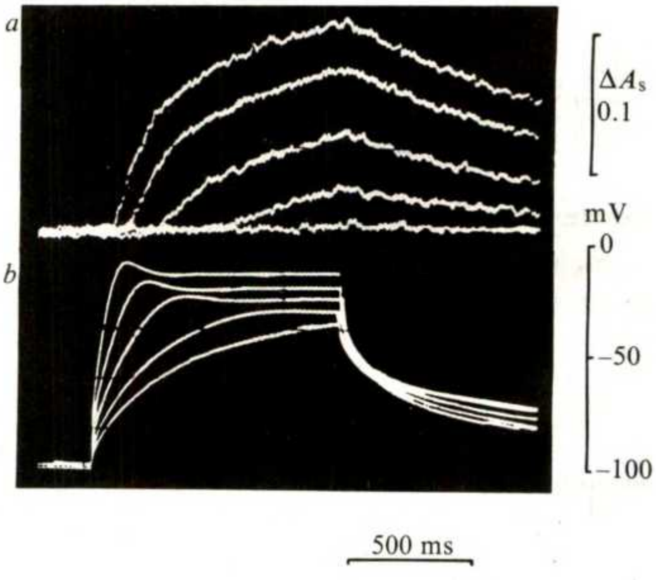


Fig. 1 Light absorbance records from a fibre injected with arsenazo III, showing responses to depolarising pulses of different amplitudes. *a*, Light absorbance changes at the wavelength pair 532–602 nm. *b*, Membrane potential. The light absorbance traces were recorded through a low-pass filter of time constant 10 ms, and the calibration (ΔA_s) gives the change in absorbance as a fraction of the total absorbance of the injected dye at a wavelength of 532 nm. An upward deflection indicates an increase in free calcium concentration, and ΔA_s of 0.1 is equivalent to an increase in free calcium of 2 μM (ref. 9). Five superimposed sweeps are shown. Stimuli were constant current pulses of 1 s duration and varying amplitudes. Temperature 7 °C.

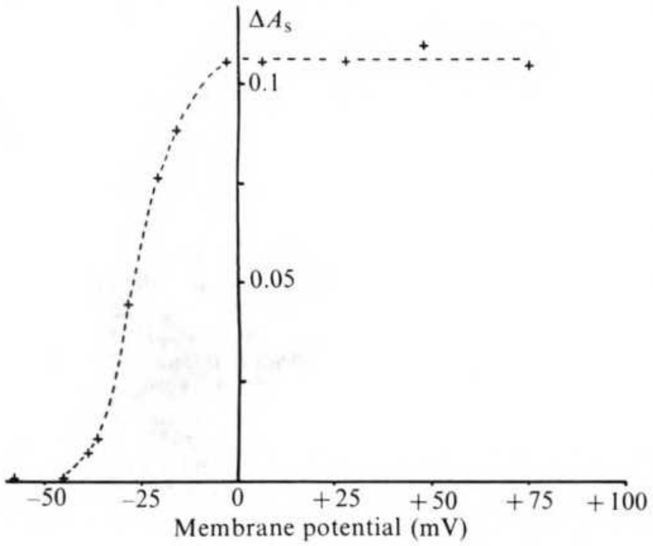


Fig. 2 Relationship between calcium response and membrane potential recorded in one fibre using depolarising pulses of 100 ms duration. Abscissa: membrane potential during pulse. Ordinate peak change in standardised light absorbance (ΔA_s) at the wavelength pair 532–602 nm. The fibre was voltage clamped using the dye pipette as a current-passing electrode, and the membrane potential was held at −90 mV between pulses. Temperature, 7 °C.

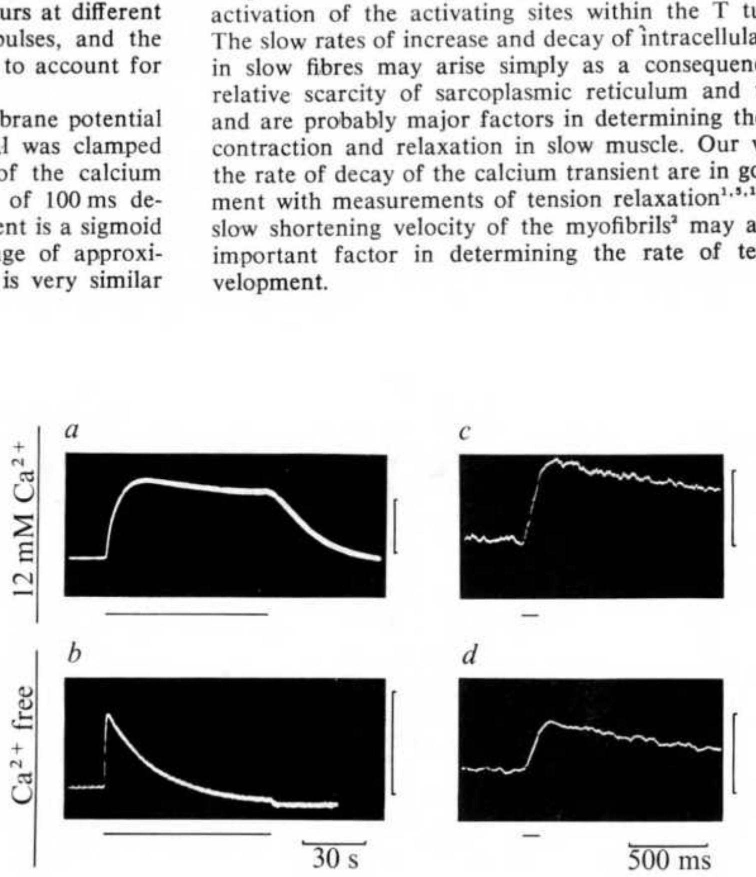
±0.025 (mean ±s.d.), when depolarised to a potential above that required to obtain a maximal response (compare Fig. 2). Corresponding values for twitch fibres are 50 ms and 0.95 μM ms^{-1} (ref. 9). The rise in calcium concentration during depolarisation shows two phases: an initial rapid increase, followed by a transition to a much slower rate of rise. This is probably a property of the calcium release mechanism, and is unlikely to be due to any nonlinearity in the recording system as the transition occurs at different calcium concentrations for different sized pulses, and the rate of calcium uptake is probably too slow to account for the transition.

To examine the relationship between membrane potential and calcium release the membrane potential was clamped at the measuring spot, and the peak size of the calcium transient measured for different amplitudes of 100 ms depolarising pulses (Fig. 2). The calcium transient is a sigmoid function of membrane potential over a range of approximately −45 to 0 mV, and the relationship is very similar to that found in twitch fibres[9]. With large depolarisations (+75 mV) the calcium response is not depressed, which suggests that the entry of external calcium is not important in the generation of the calcium transient, since the driving force for calcium entry would be reduced at this potential[9,12].

The role of external calcium was further studied by buffering the calcium concentration in the bathing solution at $<10^{-7}$ M with 1 mM EGTA and 5 mM $MgCl_2$. We found no changes in the calcium transients produced by 100-ms depolarising pulses in this solution: mean response sizes produced by supramaximal depolarising pulses in 12 mM calcium and calcium-free solution were respectively 2.0 μM ±1.1 ($n = 9$) and 1.4 μM ±0.4 ($n = 4$), while the relationship between membrane potential and calcium release and uptake remained the same (Fig. 3*c*,*d*). During a prolonged depolarisation, however, the calcium response is considerably affected by removal of calcium from the bathing solution, and the response becomes transient, rather than maintained (Fig. 3*a*, *b*). This effect is well known from studies of contractile tension[13], and might result from either an action of external calcium on the activating sites in the T-tubule membrane, or from the absence of a calcium influx which is required to maintain the intracellular calcium level. To test the importance of calcium influx during maintained contractures in 12 mM calcium, we gave a step depolarisation to +80 mV during prolonged depolarisations to +20 mV. This additional depolarisation should have reduced any calcium influx, but all experiments showed a small rise in intracellular calcium concentration, rather than a fall.

These experiments are in agreement with other work[5] in demonstrating than an influx of external calcium is not directly involved in contractile activation in slow fibres, and the striking similarity in the relationships between membrane potential and calcium response for slow and twitch fibres supports the view that activation in slow fibres occurs by the same mechanisms as in twitch fibres[4,5,13]. External calcium is, however, important in the maintenance of prolonged contractures, possibly by affecting the rate of inactivation of the activating sites within the T tubules[13,14]. The slow rates of increase and decay of intracellular calcium in slow fibres may arise simply as a consequence of the relative scarcity of sarcoplasmic reticulum and triads[15–17], and are probably major factors in determining the rates of contraction and relaxation in slow muscle. Our values for the rate of decay of the calcium transient are in good agreement with measurements of tension relaxation[1,5,18], but the slow shortening velocity of the myofibrils[2] may also be an important factor in determining the rate of tension development.

Fig. 3 Effects of removal of external calcium on the calcium responses to prolonged (*a* and *b*) and short (*c* and *d*) depolarising pulses. In each record the fibre was depolarised from −100 mV to +20 mV during the times indicated by the bars, and all records were obtained from different fibres. Ringer solution containing 12 mM $CaCl_2$ (plus 120 mM NaCl and 2 mM KCl) was used in *a* and *c*, while in *b* and *d* the solution was (mM); NaCl, 120; KCl, 2; $MgCl_2$, 5; EGTA, 1; *p*H 7. Traces show changes in light absorbance at the wavelength pair 532–602 nm, and the calibration bars are $\Delta A_s = 0.5$ for *a* and *b*, and $\Delta A_s = 0.1$ for *c* and *d*. In *b* a small shift of the baseline occurred due to movement of the fibre. Temperature, 7.5 °C.



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Induction of experimental allergic neuritis with a peptide from myelin P_2 basic protein

EXPERIMENTAL allergic neuritis (EAN) is an autoimmune demyelinating disease of the peripheral nervous system (PNS) produced in animals by injection of homogenates of PNS tissue[1] or PNS myelin[2] emulsified with Freund's complete adjuvant. Although it has been produced in such species as rabbits[1], guinea pigs[3], and mice[3], the neuritogenic determinant has not been identified. In experimental allergic encephalomyelitis (EAE), the central nervous system (CNS) counterpart of EAN, the encephalitogen is the myelin basic protein[4]. PNS myelin has two basic proteins, P_1 and P_2[5], and the latter has been suggested to be the neuritogen[2]. In attempts to induce the disease with intact P_2 (refs 6–9), mild symptoms of EAN were occasionally produced, but these were inconsistent. P_2 was implicated as the neuritogen when circulating lymphocytes of animals with EAN induced by injection of whole PNS myelin[10] were found to be sensitised to P_2 but not P_1. Physicochemical studies of P_2 have demonstrated a very stable β structure in aqueous solution[11]. Because the conformation is likely to differ in solution and in intact myelin, it is likely that the r ogenic determinant(s) are altered. We have, therefore, teste ides derived from the protein in order to minimise alter n of any neuritogenic determinant by extraneous port s of the intact molecule. We have identified a neuritogenic determinant within P_2—within a peptide representing the NH_2-terminal 21 amino acids of P_2.

P_2 (55 mg) was digested with cyanogen bromide (CNBr) (ref. 12) (in 3.0 ml 70% formic acid) and the resultant peptides were separated by chromatography on a column (1.5 × 69 cm) of Sephadex G-50. The column was equilibrated and eluted with 0.1 M acetic acid at 35 ml per h; 3.0 ml fractions were collected. Two main fragments were isolated and designated fragment I (fractions 10–35) and fragment II (fractions 38–43). Fragment II had a blocked NH_2-terminal and 21 amino acids. Fragment I comprised the bulk of the remaining molecule. The isolation and characterisation of the CNBr peptides will be described in detail elsewhere.

When tested for disease in rabbits, fragment II produced histological lesions characteristic of allergic neuritis—perivascular infiltration of mononuclear cells and demyelination (Table 1). The two rabbits receiving 300 µg each of fragment II did not develop clinical symptoms. Two of the three receiving 500-µg doses developed a general weakness and weight loss while the third developed marked clinical symptoms including ataxia and severe weakness of the hind quarters. The symptoms in this rabbit and the histological lesions in all the affected rabbits were similar to those observed when either whole PNS homogenates[1] or purified PNS myelin[2] is the inducing agent. The CNS remained essentially unchanged in these animals. Occasional lesions were observed in the spinal cord in the area of the root entry zones but these were interpreted as infiltration of cells responding to PNS myelin and overflowing into the adjacent spinal cord tissue.

Rabbit OR4 (Table 1) also had a few lesions along the ventral fissure of the lumbar cord. This animal had such an overwhelming inflammation and acute infiltration of cells throughout the PNS, especially in the dorsal root ganglia, that some spinal cord involvement extending beyond the area of the root entry zone was not surprising. Because this was the only area involving CNS tissue and was mild in comparison with the PNS involvement, it was interpreted as a nonspecific consequence of the cell-mediated response to the neuritogen, a phenomenon similar to that reported by Wisniewski and Bloom[14].

When fragment I was injected into rabbits (1 mg per animal), some histological lesions were observed, suggesting the possibility of additional neuritogenic determinant(s) in the remainder of the molecule. This latter observation is being further investigated.

Thus it seems that P_2 contains at least one determinant for EAN in the rabbit. Apparently this neuritogenic determinant is inaccessible in the isolated intact protein, probably as a result of the non-native β structure. When removed from the bulk of the protein, fragment II must assume a conformation which approximates that found in the intact myelin.

Since P_1 can also cause disease[8] the question of contamination of P_2 or fragment II with the encephalitogenic P_1 protein must be considered. In our hands, when rabbits are sensitised with P_2, no histological lesions are noted in either the CNS or PNS and only rarely are a few transitory clinical symptoms observed[7]. Thus, our P_2 preparation does not seem to be contaminated with P_1. It is even

Table 1 Induction of EAN* in rabbits by fragment II†

Animal no.	D (ı	Clinical symptoms (day of onset)	Day of killing	Histology‡			
				Perivascular cuffing		Demyelination	
				CNS	PNS	CNS	PNS
R 30	300	—	27	0	+/−	0	+/−
R 31	300	—	27	0§	+	0	+
OR 4	500	Weight loss, ataxia, and hind quarter weakness (19)	20	+/− ‖	+ +	+/− ‖	+
OR 8	500	Weight loss and weakness (23)	28	0	+	0	+
OR 9	500	Weight loss and weakness (21)	28	0	+	0	+

*Antigen was dissolved in saline and emulsified with an equal volume of Freund's complete adjuvant (H37 ra) containing 1 mg ml^{-1} *Mycobacterium tuberculosis*. Each rabbit received 0.1 ml in each of its four footpads (total 0.4 ml per animal).

†Fragment II, the NH_2-terminal 21 amino acid peptide from bovine peripheral nerve myelin P_2 basic protein.

‡The system used to grade the histology is similar to that of Alvord and Kies[12]; the Signs used are: 0, no abnormality; +/−, a few small foci of perivenular leukocytes within neuraxis; + +, large and numerous foci of perivenular leukocytes with neuraxis.

§Two foci of a few mononuclear cells in the white matter of the lumbar cord were observed.

‖Refers to entire neuraxis. There were also some + + lesions in the ventral fissure of the lumbar spinal cord with subpial demyelination. These lesions occasionally extended into the deep white or grey matter and were also demyelinative.

more unlikely that the fragment II preparation isolated from P_2 is contaminated since it is processed further after CNBr cleavage by column chromatography and/or paper electrophoresis.

EAN is the experimental disease model for one of the most common human peripheral neuropathies, infectious polyneuropathy of Guillain-Barré. The experimental disease is very similar in every regard to the human disease[15] and cells sensitised to P_2 have been found in patients suffering from this disorder[16]. Any understanding and/or treatment of the experimental disorder may prove extremely useful in devising potential treatment for the human disease.

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Myelination of mouse axons by Schwann cells transplanted from normal and abnormal human nerves

In mouse nerve iso- and allografts, Schwann cells transplanted from the donor animal survive, multiply within the graft and, eventually, ensheath axons regenerating from the host nerve[1-3]. This demonstration of the persistence of donor cells in nerve grafts has made possible the *in vivo* study of cell interactions in genetically-determined animal neuropathies. By combining normal and abnormal nerves, each originating from different strains of mice, a primary Schwann cell abnormality has been demonstrated in Trembler[3] and Quaking[4] mutant mice while axonal factors seem to be responsible for changes in the spinal roots of dystrophic mice[5,6]. We report here the successful transplantation into immunologically-suppressed mice of human Schwann cells from control patients and from the sural nerve of a child with metachromatic leukodystrophy. Metachromatic leukodystrophy (MLD) is a human autosomal recessive disease due to aryl sulphatase deficiency and characterised by degeneration in the central and peripheral nervous systems with intracellular accumulations of metachromatic granules[7,8].

Control human sural nerves were obtained from limbs amputated because of peripheral vascular disease or trauma and from a diagnostic biopsy taken from a 10-yr-old child investigated for muscle weakness. Sections obtained from these control nerves showed no evidence of neuropathy by light microscopy. Segments of MLD nerve were obtained from a sural biopsy of a 10-yr-old girl with a 5-yr history of progressive neurological deterioration and peripheral neuropathy. The diagnosis of MLD was documented by two nerve biopsies, both of which showed the characteristic metachromatic material within Schwann cells.

Fascicles 5 mm in length were removed from the control human nerves and, in 23 normal C57 BL/6J mice, grafted between the stumps of left sciatic nerves transected at mid-thigh level. Nerve from the patient with MLD was grafted in identical fashion into 12 other C57 BL/6J mice. All grafts were secured by 10-0 nylon sutures placed through the connective tissue surrounding the nerve. These procedures were carried out in aseptic surgical conditions within 3 h of limb amputation or biopsy, donor nerves being kept in cold Ringer's solution before grafting.

To prevent rejection of the xenografts, anti-lymphocytic serum (ALS) was prepared by injecting rabbits twice with a suspension of 10^9 living thymocytes from C57 BL/6J mice[9] and administered subcutaneously to the host animals in doses of 0.5 ml twice weekly. At intervals from 10 days to 3 months after grafting, host mice were killed by systemic perfusion of fixative containing 1.5% glutaraldehyde, 0.05% formaldehyde and 0.5% calcium chloride in 0.08 M Sorensen's phosphate buffer. Cross sections from the proximal and distal sciatic nerve stumps of the host and from the mid-portion of the graft were examined by phase and electron microscopy.

Soon after grafting, nerve fibres in the graft and distal stumps of the grafted nerves underwent characteristic changes of Wallerian degeneration but there was no rejection of the graft. From 3 weeks after grafting, there were increasing numbers of regenerated myelinated and unmyelinated nerve fibres within the graft and distal nerve stumps (Fig. 1*a*, *b*). Although the initial stages of regeneration and myelination were similar in grafts originating from control and MLD nerves, numerous metachromatic granules were observed within Schwann cells in the MLD grafts by 2½ months after grafting (Fig. 2*a*, *b*).

Schwann cells in the grafted segments of these regenerated nerves could have originated either by migration from the proximal nerve stump of the host or from Schwann cells indigenous to the graft. In the first instance, Schwann cells in the regenerated graft would be mouse Schwann cells; in the second, human Schwann cells would ensheath mouse axons. Although large numbers of Schwann cells do not migrate from the proximal stumps of host nerves into mouse isografts or allografts during regeneration[1-3,10], it is conceivable that these cells might behave differently in xenografts. Thus, to identify cells of mouse and human origin in these regenerated nerves, ALS was discontinued

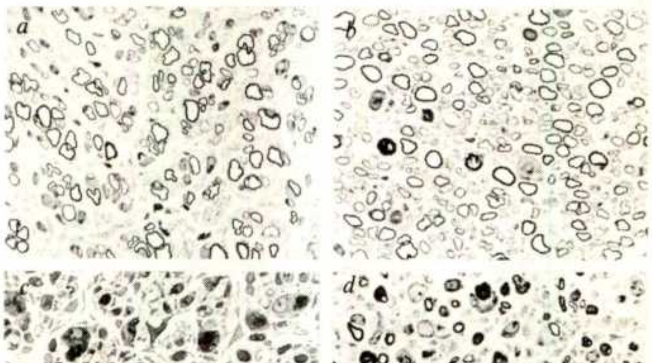

Fig. 1 Cross section of grafted segments of distal stumps of human sural nerve to mouse sciatic nerve xenografts. *a, b,* 10 weeks after grafting a segment of control human sural nerve into the sciatic nerve of an immune-suppressed mouse, axons have regenerated from the proximal stump into the graft and distal stump. At both these levels of the same regenerating nerve, there are abundant myelinated fibres. *a,* Graft; *b,* distal stump. 4 weeks after stopping ALS and 10 d after the transfer of immune cells to the host animals, a 6-week-old regenerated graft is rejected. The rejection reaction is indicated in the graft (*c*) by myelin debris, increased cellularity and a complete loss of myelinated fibres. In the distal stump of the same nerve (*d*) many myelinated fibres are preserved although some show signs of Wallerian degeneration, presumably due to axonal damage at the level of the graft. (Phase contrast photomicrographs (× 660).

in a group of mice from 4 weeks to 2 months after grafting. From 2 to 3 weeks after discontinuation of ALS these mice also received intravenously 10^8 lymphoid cells prepared from the spleen and lymph nodes of syngeneic mice hyper-immunised against tissue antigens by intravenous injection of 10^7–10^8 human leukocytes twice weekly for 3 weeks. This cell transfer was used to abrogate the xenograft acceptance in the recipient animal and to ensure a more rapid rejection of all 'foreign' cells[11]. Although discontinuation of ALS will cause xenograft rejection, the additional immune cell transfer permits a more accurate timing of rejection for histological studies.

Ten mice in which ALS had been discontinued were examined for 4–81 d after immune cell transfer. On d 8, early rejection was indicated by small accumulations of mononuclear cells in the nerve grafts. By d 10, there was an intense reaction in the grafted segments (Fig. 1*c*). Schwann cells of both myelinated and unmyelinated fibres were surrounded, penetrated and eventually replaced by mononuclear cells (Fig. 3*a*). Perineurial fibroblasts were also rejected in the grafts. During this acute phase of rejection, some axons became totally denuded of Schwann cells and were surrounded only by basal lamina (Fig. 3*b*).

During graft rejection, many fibres in the distal stumps remained myelinated, showing that the continuity of their axons was preserved. But, a certain number of axons must have been interrupted, presumably in the graft, because fibres undergoing Wallerian degeneration were observed in distal stumps of the same nerves (Fig. 1*d*).

In two animals allowed to survive the period of rejection for more than 2 months, the segments of nerves at the site of the original graft again contained large numbers of myelinated fibres. Presumably, migration of mouse Schwann cells from the stumps of the host nerves was responsible for the reappearance of these myelinated fibres. This migration may have been facilitated and guided by the axons and empty basal lamina which survived rejection.

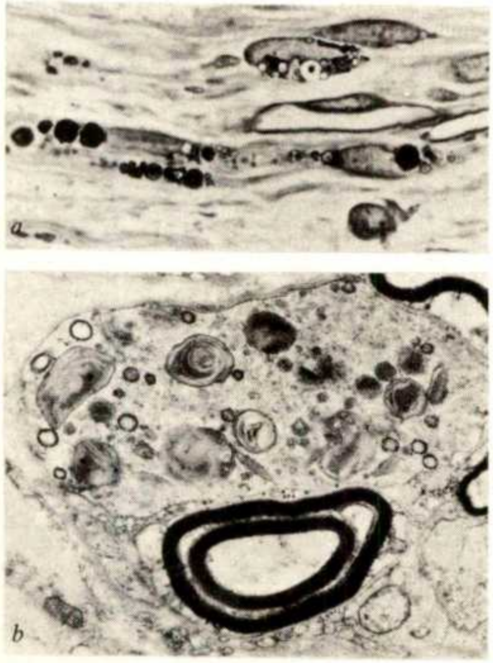

Fig. 2 Segments of human MLD sural nerve grafted into mouse sciatic nerve. *a*, After 2½ months, axons from the mouse nerve stump have regenerated into the graft and are associated with Schwann cells which contain myelin ovoids and numerous small granules. These granules, which stain metachromatically with cresylviolet, were confined to the graft and were not observed in the proximal and distal nerve stumps. (Longitudinal section, phase micrograph (× 1,200). *b*, Lamellated and amorphous intracellular inclusions are present in the mid-portion of a human MLD to mouse sciatic nerve graft. (Electron micrograph (× 13,500).

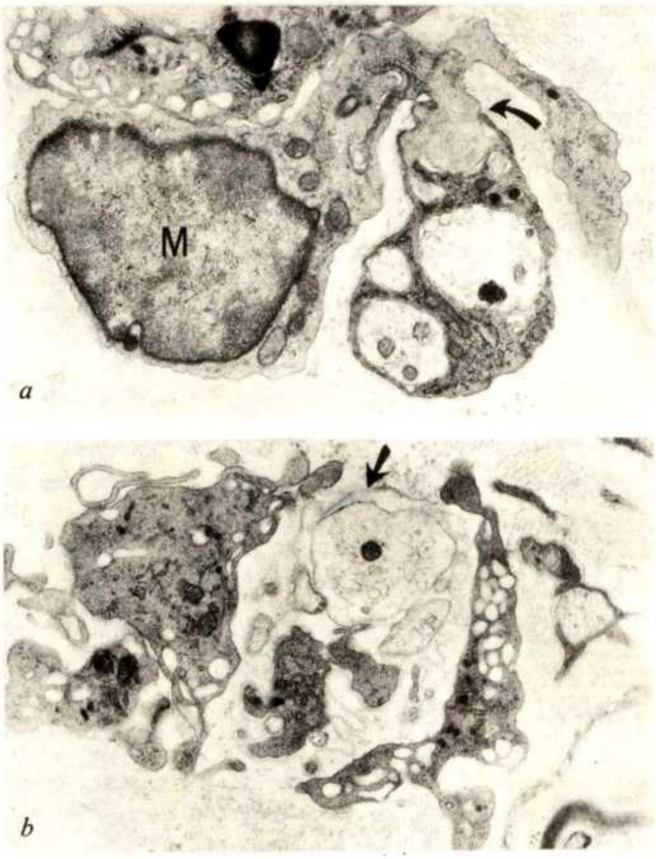

Fig. 3 Details of rejection of the nerve graft shown in Fig. 1*c*. A cytoplasmic process from a mononuclear cell (M) has partially penetrated the basal lamina of an unmyelinated fibre (arrow). *b*, One large and several small axons persist within the basal lamina (arrow) of a rejected Schwann cell. Macrophage processes are present on both sides of the residual basal lamina. (Electron micrograph (× 9,800).

The selective rejection of most of the sheath cells in the regenerated grafts establishes that the grafted segments of these nerves still contained fibroblasts and Schwann cells foreign to the mouse. Thus, regenerated nerve fibres within the grafts must represent a combination of mouse axons and human Schwann cells. The presence of metachromatic granules in grafts originating from the sural nerve of a patient with MLD 2½ months after grafting into mouse nerves is additional evidence of the presence of human Schwann cells in the regenerated xenografts. This finding also suggests that the grafted MLD sheath cells continue to be deficient in aryl sulphatase during nerve regeneration in the mouse and may be unable to utilise this enzyme from the host.

These studies in nerve xenografts have shown that (1) human Schwann cells can ensheath and myelinate regenerating mouse axons; (2) the perineurium of the regenerated grafts contain human fibroblasts; (3) during rejection of regenerated grafts, the immune response is directed against fibroblasts as well as Schwann cells of myelinated and unmyelinated fibres; axons may be affected secondarily[12]; (4) if, after regeneration across a graft, the xenogenic sheath cells are rejected, they can be replaced by the host's own Schwann cells which migrate along the re-established pathways formed by axons and basal lamina tubes; (5) human neuropathies may be reproduced by transplanting fascicles from the patient's nerve into immune-suppressed animals.

Thus, the present *in vivo* combinations of human and animal cells may help in the investigation of biological mechanisms and permit a better definition of the role of axons and Schwann cells in normal and pathologic human nerves. Human Schwann cells transplanted into laboratory animals are now amenable to experimental manipulations which may include enzyme replacement and immunological studies.

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Two mechanisms for positive inotropism of low-K Ringer solution in bullfrog atrium

IT has long been known that a reduction of K concentration in external fluid immediately potentiates the twitch contraction of frog heart[1]. Microelectrode studies[2,3] have revealed a hyperpolarisation of the membrane and a prolongation of the action potential, which was thought to be a cause of the potentiation of contraction. But, when the muscle is soaked in K-depleted fluid the membrane Na/K ATPase and the activity of the Na pump are inhibited[4] and Na ions progressively accumulate within the cell[5]. In myocardium, the accumulation or rise of $[\mathrm{Na}]_i$ produces a secondary increase of $[\mathrm{Ca}]_i$ due to Na–Ca exchange diffusion and the consequent positive inotropic effect[6,7]. Thus, Ehara[8] has demonstrated the presence of a delayed potentiating effect of low-K Ringer solution possibly due to Na-pump inhibition in the frog ventricle as well as its immediate inotropic effect. We have tried to clarify further the nature of these inotropic actions. We show here that, in the frog atrium, augmentation of Ca-inward current (I_{Ca}) and increase of I_{Ca}-independent tonic tension, related to Na–Ca exchange, are responsible for the immediate and delayed inotropic effects under voltage clamp, respectively.

Thin muscle bundles (diameter 0.4–0.5 mm) isolated from the right atrium of the bullfrog, *Rana catesbiana,* were used. The membrane potential, current and tension were measured in voltage-clamped and unclamped conditions with the conventional double-gap method[9], in which the width of the central test chamber was about half (0.3–0.4 mm) of the space constant[10]. With this apparatus gap action potentials (AP) of more than 100 mV were commonly obtained and the short circuiting factor was greater than 0.8 for both gaps. The central chamber was initially perfused with normal Ringer containing 2.5–4.0 mM K until the twitch tension became steady, and then the solution was replaced with low-K Ringer (0–0.16 mM K). All experiments were performed at a constant temperature, 16–17 °C.

In unclamped conditions, K-depleted Ringer solution produced biphasic positive inotropic effects in some atrial preparations (Fig. 1*a*) but not as often as in the ventricle[8]. The partially fused effects seemed much faster (five to six times) in the former than in the latter, probably due to thiness of the muscle fibres and scattering of the bundles in the atrium. The immediate and late potentiations, however, could be discriminated in the presence of ouabain (10^{-6} M, Fig. 1*c*) which eliminated the late potentiating effect. The initial inotropic effect was accompanied by a hyperpolarisation of the membrane, a rise in threshold, and an increase in amplitude and duration of AP. During the late inotropic effect, on the other hand, a gradual depolarisation plus lowering and prolongation of AP were produced together with a gradual increase of membrane slope resistance (Fig. 1*d*). Automaticity was often elicited during this later phase (Fig. 1*b*), and augmented twitch contraction showed a special pattern of fast tension development and slow relaxation. In the final depressive stage of contraction, the basal tension increased and the AP was further suppressed; and after a few hours of soaking in K-depleted Ringer, addition of K ions (4 mM) produced recovery, and hyperpolarisation instead of depolarisation of the membrane. This suggests activation of the electrogenic Na pump (Fig. 1*e*).

Voltage-clamp studies during the initial 2–5 min after K-depletion disclosed that considerable potentiation of contraction still occurred in response to constant depolarising pulses. The fast Na-inward current (I_{Na}), though not accurately measurable, was virtually unchanged during this period (see Fig. 2*c*), however the slow inward current in the presence of tetrodotoxin (TTX) ($5\times10^{-7}\,\mathrm{g\,ml^{-1}}$) and the Ca-inward current (I_{Ca}) isolated in Na-free sucrose Ringer solution were markedly prolonged after K-depletion. The

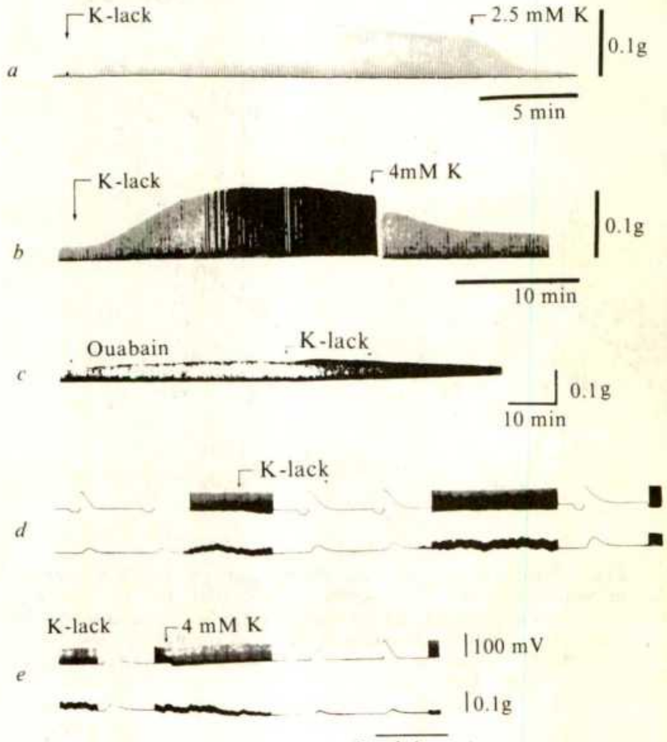

Fig. 1 Immediate and delayed positive inotropic effects of K-free Ringer on bullfrog atrium. *a,* Biphasic inotropic responses. *b,* Monophasic inotropic response with arrhythmia at later phase. *c,* Effects of K-depletion in the presence of ouabain (10^{-6} M). *d,* Effects of K-depletion on membrane potential and contractility. A hyperpolarising pulse was given before each action potential. *e,* Effects of administration of m 4 mM K at the depressive stage 5 h after K-depletion. Note increase of membrane slope resistance and hyperpolarisation followed by gradual depolarisation in (*c*) and appearance of hyperpolarisation after administration of K in (*e*).

prolongation was mainly due to a long delay of the inactivation process (Fig. 2*a*,*b*). The leaky inward and outward currents decreased as expected (Fig. 2*c*). But, the voltage–current relationships obtained in Na-free Ringer solution, in which the anomalous rectification was completely eliminated, confirmed the initial enhancement of I_{Ca} after K-depletion (Fig. 2*d*). The experiments also clarified a concomitant augmentation of the I_{Ca}-dependent tension (not shown). These observations clearly indicate that the initial inotropic effect of K-depletion is not simply dependent on prolongation of the AP due to decrease of leaky currents but also on an augmentation of I_{Ca} and I_{Ca}-dependent tension.

Meanwhile, during the late inotropic phase more than 10 min after K-depletion, the fast and slow inward currents were gradually depressed (not shown). The I_{Ca}-dependent phasic component of tension diminished, while the I_{Ca}-independent tonic component was markedly augmented (Fig. 3*a*). Voltage–current relationships during these later phases also showed a decrease of the slow inward current (Fig. 3*b*) as well as the isolated I_{Ca} (Fig. 2*d*). Depression of the leaky inward and outward terminal currents persisted. Those characteristics may well account for the typical configuration of the action potential: depressed overshoot and plateau followed by a long tail (Fig. 1*e*). The marked reduction of electrogenic outward current by inhibition of Na pump observed in sheep Purkinje fibres[11] was not seen in the frog atrium, although a presence of an electrogenic potential was noticed in the final depressive stage of contraction. Thus, the principal cause of the late positive inotropic effect of low-K solution was augmentation of I_{Ca}-independent tonic tension and prolongation of the action potential.

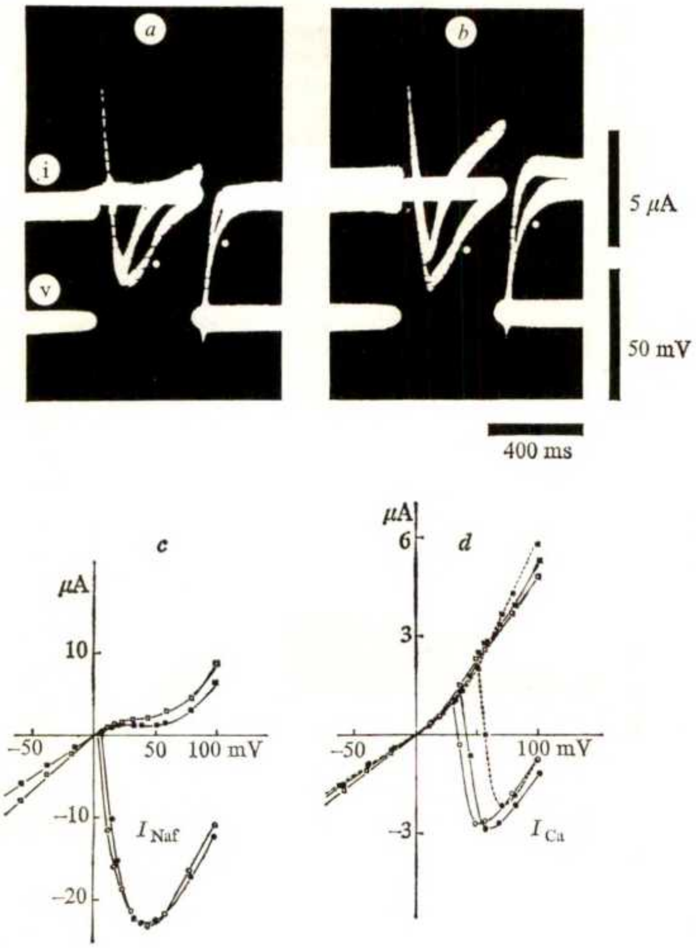

Fig. 2 Initial effects of K-depletion on membrane currents in voltage clamp. The membrane potential was held at the gap resting potential and rectangular pulses were applied at intervals exceeding 10 s. *a*, Superimposed records of Ca-inward currents before, and 5 min after K-depletion (○) in response to the same depolarising pulses (47 mV, 400 ms) given from the holding potential level (−70mV). Data were obtained in Na-free sucrose Ringer solution throughout. *b*, Similar superimposed records taken 6 min after K-depletion (○) and 2 min after washing with normal Ringer solution (4 mM K). Note prolongation of Ca inward current after K-depletion in (*a*,) and depression of current after washing in (*b*). Similar changes are observable in the tail currents. *c*, Voltage–current relationships before and after K-depletion. Long depolarising pulses (1.0 s) were used. ○, □, Control. ●, ■, 10 min after K-lack *d*, As (*c*), but in Na-free sucrose Ringer solution. ○, □, Control. ●——■, 3 min after K-lack. ● . . . ■, 10 min after K-lack. In (*c*), both inward and outward terminal currents (squares) were markedly inhibited while fast dynamic inward currents (○) were unaltered. In (*d*), no anomalous rectification was observable due to Na-free condition. Note apparent enhancement of I_{Ca} (○) at 3 min after K-depletion.

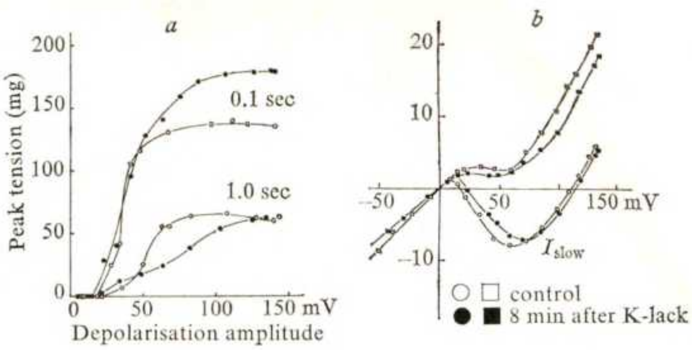

Fig. 3 Late effects of K-depletion on membrane currents and tension components under voltage clamp. *a*, Voltage-tension relationships. Short and long depolarising pulses (0.1 s and 1.0 s) were used and peak tension before (○), and 10–15 min after (●) K-depletion were plotted against amplitude of pulses. The contractile tensions in response to short pulse (mainly I_{Ca}-dependent tension) were inhibited while those for long and strong pulses (largely I_{Ca} -independent tension) were augmented. *b*, Voltage–current relationships before (○, □) and 8–10 min after (●, ■) K-depletion in presence of tetrodotoxin (5×10^{-7} g ml^{-1}). The squares mark terminal currents and the circles, peak inward currents. Long pulses (1.0 s) were used. The slow inward current and the inward as well as outward terminal currents were depressed.

It should be noted that these initial and late effects of K-depletion seemed to be quite similar to those of ouabain[12,13]. Although the mechanism of therapeutic action of ouabain is not yet known, one possibility suggested by Baker *et al.*[14] and Langer[15] is that inhibition of the Na pump and the consequent increase of $[Na]_i$ may allow an accumulation of Ca into the cell through the Na–Ca exchange mechanism. In fact, ouabain was shown by Vassort[16] to increase only the I_{Ca}-independent tonic tension in frog atrium, which largely depends on Na–Ca exchange. This may be compatible with the late inotropic effect of K-depletion in our case where the Na pump was also inhibited[8]. Another possibility has been proposed[17,18], however—a therapeutic low dose of ouabain may produce net stimulation rather than inhibition of the Na/K-ATPase. Blood[19] used doses of ouabain (5×10^{-10} to 10^{-7} M) that exert a definite positive inotropic action on Purkinje fibre. These doses were found by Cohen *et al.*[20] to increase the K gradient across the membrane, probably as a consequence of stimulation of the Na pump. These effects may well correspond to the initial effects of K-depletion, at least in the condition in which $[K]_0$ decreases. The inotropic action of cardiac glycosides is probably not mediated by an increase of I_{Ca}, but rather, is accompanied by its decrease[12,13]; however, the effects of a low dose on I_{Ca} have not been examined. In any case, the appearance of I_{Ca} augmentation after K-depletion may explain some part of the long known antagonistic action of Ca and K on myocardial contractility.

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Sarcomere shortening in striated muscle occurs in stepwise fashion

During the development of a high resolution light diffractometer to study sarcomere dynamics, we have observed an unexpected feature of the pattern of sarcomere shortening. We report here that sarcomere shortening during contraction seems to occur in a series of successive bursts punctuated by short but well defined periods during which motion has virtually ceased. Furthermore, it seems that this stepwise pattern of shortening may occur synchronously over many contiguous sarcomeres.

Cardiac and skeletal muscle specimens were mounted in an apparatus (Fig. 1), components of which have been described[1]. The muscle was transilluminated with laser light (Spectra Physics He-Ne laser, Model 120, $\lambda = 0.6238\ \mu$m). The diffraction pattern was collected at the rear focal plane of a water immersion objective (Zeiss ×40, effective numerical aperture 0.5). Sarcomere length was computed from the spacing between diffracted orders using the Fourier relationship.

We use three methods of analysis (Fig. 1). With each method the diffraction pattern was first compressed along the length of each order with a cylindrical lens; this increased intensity and averaged optical noise. The compressed pattern was then projected on the three sensors. The principal sensor was a 128-element photodiode array (Reticon RL-128A; diode spacing 50 μm; aperture 25 μm × 430 μm). The array was scanned 5 times per ms. Each scan generates a profile of intensity against spatial frequency of the striation pattern. Sarcomere length was computed during each scan from the distance between the zeroth order peak and the computed median of the first order (T.I. and G.H.P. in preparation). The sarcomere length signal was calibrated with a series of gratings; the accuracy was within 1% between 1.6 μm and 3.3 μm. Resolution was approximately 5 nm.

In the second method a slice of the diffraction pattern was projected through a slit (width 0.9 mm) on to film in a kymographic camera[2]. The time resolution was limited to about 4 ms. Densitometry measurements were made at intervals of 2 ms along the film, and a plot of intensity against spatial frequency was obtained at each location. The position of the median of the first order was determined by planimetric integration. Sarcomere length was computed from the spacing between the median and the zeroth order peak. From these computations a plot of sarcomere length against time was obtained.

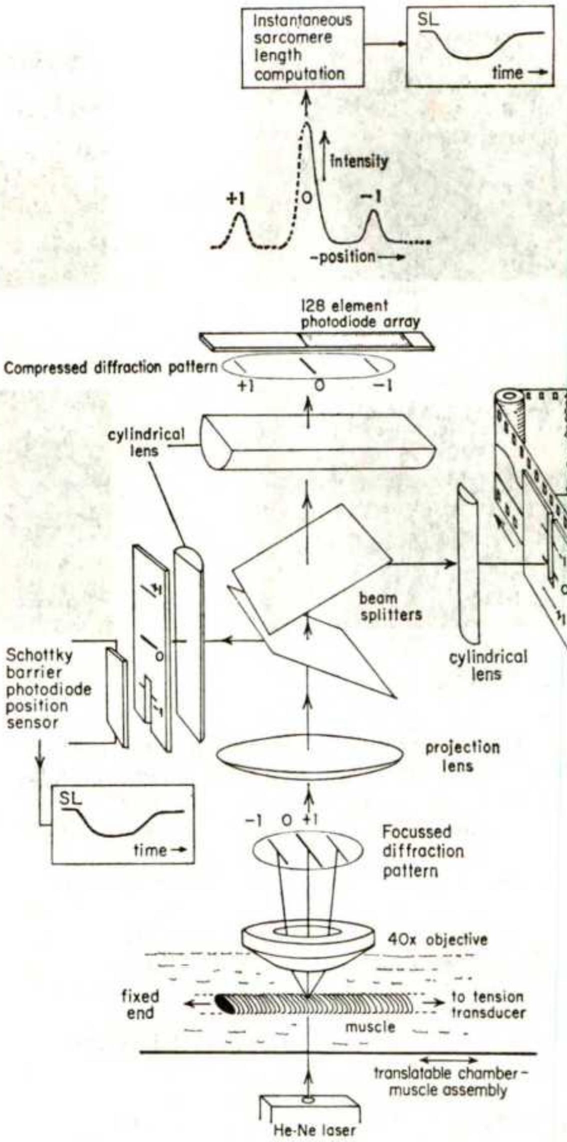
Instantaneous sarcomere length computation
SL
time →
Intensity
+1
0
−1
−position→
128 element photodiode array
Compressed diffraction pattern
+1
0
−1
cylindrical lens
beam splitters
cylindrical lens
Schottky barrier photodiode position sensor
SL
time →
projection lens
−1 0 +1
Focussed diffraction pattern
40x objective
fixed end
to tension transducer
muscle
translatable chamber-muscle assembly
He-Ne laser

Fig. 1 Apparatus used to measure sarcomere length with high time and length resolution. The diffraction pattern of the muscle collected at the rear focal plane of the objective lens is projected onto three sensors for analysis. Sarcomere length is derived from the spacing between bands of the diffraction pattern.

In the third method, one of the first orders was projected through a slit onto a Schottky barrier photodiode (United Detector Tech. SC/25). The photodiode has a cathode at each end, and the difference of cathode currents is directly related to the position of the centroid of the light spot. Sarcomere length was computed from this current difference, giving a continuous output with a bandwidth of about 100 kHz. Calibration error was within 3% throughout the relevant range of striation spacings.

Representative examples of the time course of sarcomere shortening during contraction obtained with the photodiode array are shown in Fig. 2. These are records of 'internal' shortening during isometric twitches (though comparable phenomena were observed in non-isometric twitches and

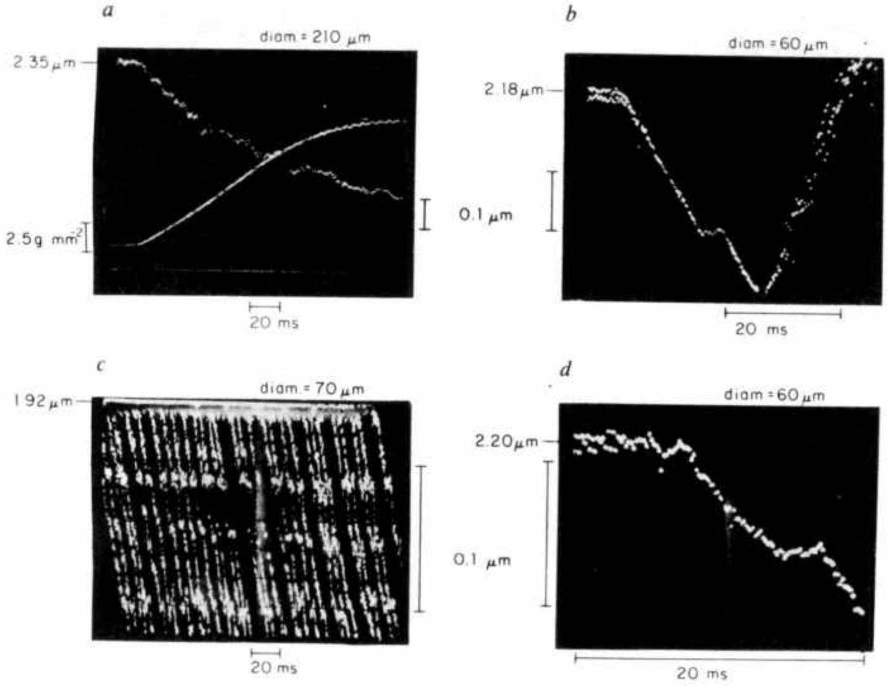

Fig. 2 Records of the time course of sarcomere length during contraction, taken from rat papillary (*a*), frog skeletal (*b* and *d*) and rat trabecular (*c*) muscle. All records were obtained using the photodiode array. In (*a*) the tension record and the stimulus pulse are shown as well. Note the stepwise shortening pattern observed early in the contraction. In the twitch depicted in (*b*) only one step is seen, lasting about 4 ms. The trace becomes noisy during relaxation as a consequence of the breakup of the diffraction pattern, as reported previously[2]. The Records in (*c*) are from a series of contractions (each displaced successively rightward) and demonstrate the repeatability of the stepwise pattern. Because of the expansion of the sarcomere length scale relative to the time scale, each plateau appears as a bright spot on the trace. Record (*d*) was obtained with high time and sarcomere length sensitivity on the oscilloscope, so that each sarcomere length determination showed up distinctly. Only the early portion of contraction is shown. The transition between shortening and plateau (right side of panel) occurs within several hundred microseconds, the time for one or two sarcomere length determinations.

tetani). In Fig. 2*a* shortening commenced after a latent period following stimulation and continued for several milliseconds. Then, abruptly, shortening ceased and did not resume for 8–10 ms (a period encompassing 40–50 determinations of sarcomere length). This stepwise shortening pattern was repeated several times during the early phase of contraction. Later in the cycle the signal became irregular and failed to show distinct plateaus. Thinner specimens often showed plateaus throughout the shortening phase of contraction, possibly because of their relatively sharper diffraction patterns.

Figure 2*b* shows a shortening pattern obtained from a single fibre of skeletal muscle. Only one distinct plateau is evident. Although other records did show multiple plateaus, we usually found fewer and shorter plateaus than in comparable contractions of cardiac muscle. The series of shortening traces in Fig. 2*c* was obtained from successive contractions in the same optical region of a trabecular specimen. As this record shows, plateaus occurred at approximately the same sarcomere length in every contraction. In different regions along the specimen, however, different stepwise shortening patterns were usually found, and in some regions no steps were evident.

Figure 2*d* shows a portion of a shortening trace recorded with high sensitivity to illustrate the abruptness of the transition between shortening and plateau. Transitions were usually complete within 1 ms.

Stepwise shortening patterns might arise artefactually from at least two potential sources. The specimen could translate across the optical field from a shortening to a static region. We have seen such patterns, however, in regions which translated only several per cent of the field diameter throughout contraction. Another potential source of artefact is the photodiode array and associated electronics. To test this, we recorded some diffraction patterns simultaneously using both the photodiode array and the kymographic technique outlined in Fig. 1.

Analysis of the kymographic records was not entirely satisfactory, as the limited time resolution precluded the delineation of any sharp corners in the shortening pattern. Furthermore, faithful representation of the shortening pattern placed high demands on the accuracy of the planimetric determinations; relatively minor errors produce enough scatter to blur fine details of the shortening pattern.

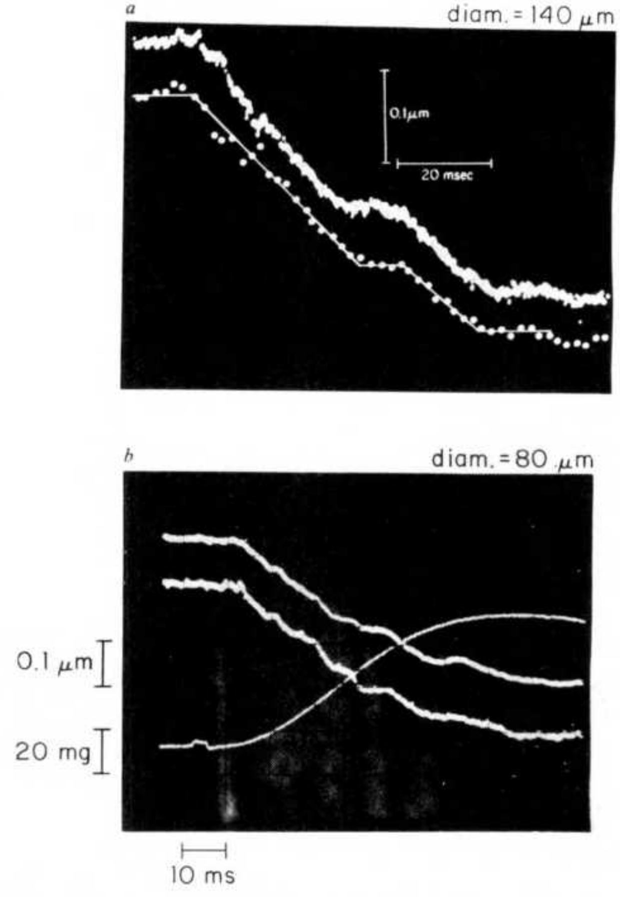

Fig. 3 Time course sarcomere length (from rat trabecular muscle) measured simultaneously with the photodiode array (continuous curve) and with the kymographic technique (points). The records are vertically displaced for clarity. Initial sarcomere length was 2.3 μm. A plateau seems to be present in both records at a point just beneath the sarcomere length scale; however, the restricted time and space resolution of the kymographic technique limits the confidence with which the existence of a plateau can be inferred. *b*, Time course of sarcomere length changes measured simultaneously with the photodiode array (upper trace) and the Schottky barrier photodiode (middle trace). Records are vertically displaced for clarity. Initial sarcomere length was 2.1 μm. The tension record, with superimposed stimulus artefact, is shown beneath.

Nonetheless, these records showed a fair degree of correspondence with those obtained with the photodiode array (Fig. 3*a*).

Because we judged the correspondence between the two results to be inconclusive, we adopted still another methodology, the Schottky barrier photodiode (Fig. 1). As Fig. 3*b* shows, the records obtained with this analogue technique show a stepwise shortening pattern similar to that obtained simultaneously with the photodiode array. The similarity of the records convinces us that the spacing between the zeroth and first order changes in stepwise fashion.

If so, the shortening behaviour among a large fraction of the sarcomeres in the optical field must have been coordinated; otherwise the sharp corners in the shortening trace could not have been observed. Since each field covered approximately 180 μm, the region of spatial coherence must have extended over at least the major fraction of this dimension. On the other hand, we found regional variations along the muscle of the step-like shortening patterns, so that the region of spatial coherence apparently did not extend far beyond the dimension of an optical field.

Plateaus in the shortening patterns could arise in at least three ways: (1) most sarcomeres in an optical field could undergo no length change during the period of the plateau; (2) populations of lengthening and shortening sarcomeres could cancel one another, or (3) all sarcomeres might shorten continually, but the contribution to the diffraction pattern of the shorter sarcomeres could diminish gradually (due perhaps to increasing misregistration of sarcomeres), as the contribution of the longer sarcomeres gradually increases.

Possibility (3) requires that at least two distinct sarcomere populations exist within the optical field, that the degree of spatial coherence of each be cyclic and out of phase with the other, and that the cycles occur at specific sarcomere lengths. The combination of these *ad hoc* assumptions seems too unrealistic to make this explanation likely. Possibility (2) demands that a fraction of sarcomeres in the field suddenly begins lengthening just rapidly enough to cancel the contribution of shortening sarcomeres. By the end of a plateau the two populations ought to have diverged by an amount detectable on the kymographic records; we observed no such divergence. Thus the first possibility—that most sarcomeres in the field suddenly cease shortening—remains the most likely explanation of the plateaus in the stepwise shortening pattern.

If our observations withstand the test of time, any proposed molecular theory of contraction would have to incorporate a mechanism to account both for stepwise shortening in a single contractile unit, and for synchronisation of this stepwise pattern among a large number of contiguous contractile units. It is not immediately obvious how the cross-bridge theory as now envisaged[3,4] might satisfy such a requirement.

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Does electrophoresis of cockroach muscle proteins detect recognition molecules?

IN some animals, particularly amphibia and insects, normal function is ultimately restored in a limb which has been denervated. In the cockroach it has been shown that identified motoneurones reinnervate the same leg muscles as they did before the nerve was sectioned[1,2]. A possible explanation is that each muscle has specific molecules by which ingrowing nerves are able to recognise their appropriate destination. Recently, Denburg[3], using sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis has shown that these cockroach muscles have distinct gel protein patterns which he suggests represent specific recognition molecules. Alternatively, the differences may arise because the motor neurones he considers are physiologically different types, 'fast' and 'slow'. To test this interpretation we have examined a number of other cockroach muscles using Denburg's methods. All the muscles have patterns which may be grouped into the same categories as Denburg's even though they are innervated by different neurones. Where the innervation by 'fast' and 'slow' neurones is known the correspondence with our data is good. Furthermore, locust flight muscles with solely 'fast' innervation all have identical patterns. We conclude that gel electrophoresis patterns demonstrate a characteristic of the type of innervation rather than representing specific recognition molecules.

On theoretical grounds it seems unlikely that a procedure as crude as SDS gel electrophoretic analysis of whole muscle extract should reveal molecules concerned in specific recognition by motoneurones. One might reasonably expect that every

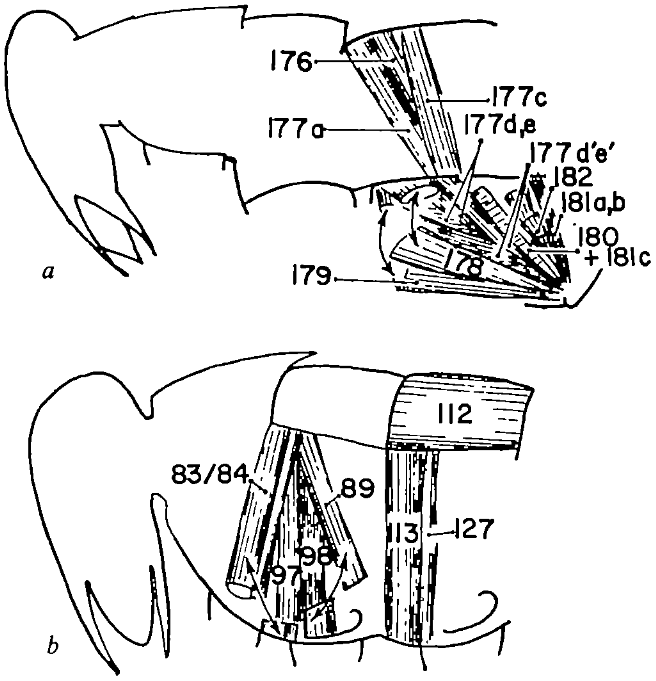

Fig. 1 *a*, Positions of the muscles in the cockroach which have been examined by SDS–polyacrylamide electrophoresis (see Fig. 2). The muscles are numbered according to Carbonell[5]. Muscles 161, 163 and 167 lie close to 177a and have been omitted for clarity. Muscle 174 lies close to 176 and is omitted also. *b*, Positions of the flight muscles in the locust which have been examined (see Fig. 3).

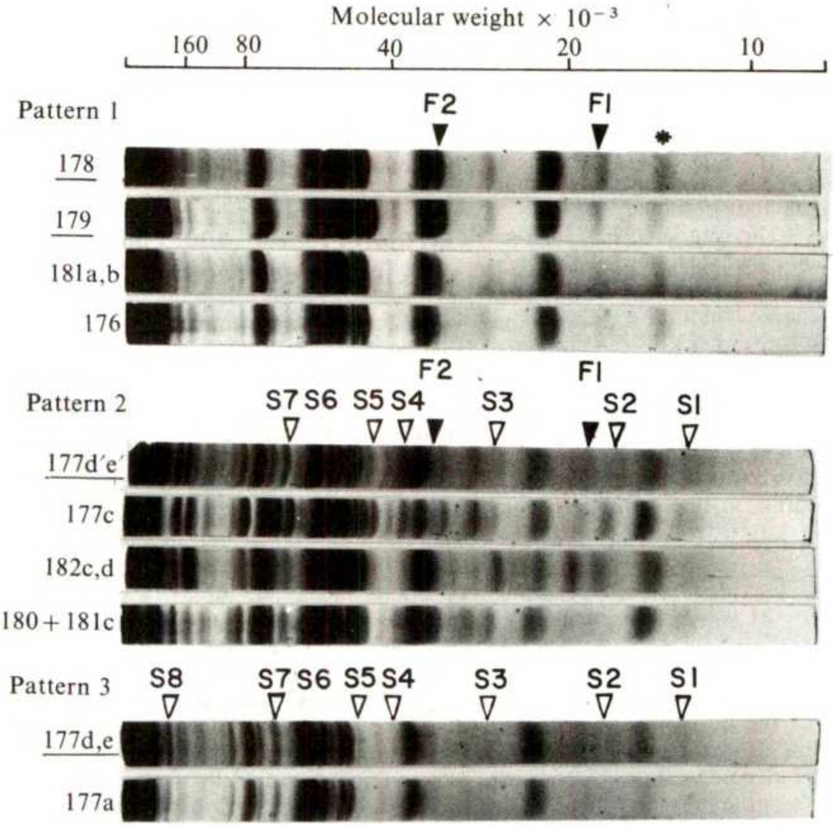

Fig. 2 SDS–polyacrylamide gels of the cockroach muscles shown in Fig. 1*a*, stained with Coomassie Blue. The numbers of the muscles examined by Denburg are underlined and those bands corresponding to the R_f values of his bands F1–2 and S1–8 are indicated. An additional band (labelled *) not described by Denburg is also indicated. This figure demonstrates that the patterns 1–3 from the muscles Denburg examined are not unique to muscles innervated by motoneurones 28 and 52, which argues strongly against their representing specific recognition molecules. Isolated muscles were homogenised in an appropriate volume of 0.01 M Tris-HCl (*p*H 7.4), 20% gylcerol, 1% SDS and 5% 2-mercaptoethanol and heated in a boiling water bath for 5 min. Aliquots of these solutions were applied to 10% polyacrylamide gels which were run in the presence of 0.2% SDS, stained with Coomassie Blue, and destained using the methods of Fairbanks *et al.*[4]. The gels have been severed at the dye marker band.

structural and soluble protein of the cell would be represented in the extract and yet the largest number of bands we have observed using Denburg's technique is 33. Clearly only those proteins with the largest representation in the cell are being made visible by the method and it would seem improbable that specific recognition molecules, which have previously evaded identification would be in the top 33.

Denburg found that the metathoracic coxal depressor muscles 178 and 179, which are innervated by cell 28, have a banding pattern different from that of muscles 177d and 177e which are innervated by cell 52. The gel patterns for muscles 177d′ and 177e′, which are innervated by both motoneurones, have features common to both types (Figs 1*a* and 2). He considers that particular bands in these patterns represent distinctive macromolecules by which ingrowing nerves are able to recognise their appropriate muscle. Our alternative explanation is that the gel protein pattern for muscles innervated by cell 28 is different from those of muscles innervated by cell 52 simply because cell 28 is a 'fast' motoneurone, while cell 52 is a 'slow' motoneurone. If this interpretation is correct then all muscles with 'fast' innervation should show the one pattern, those with 'slow' the other, and muscles innervated by both kinds of neurone should show the sum of the two patterns. Unfortunately Denburg only analysed the muscles which are innervated by cells 28 and 52 and he did not test this possibility.

We have examined 14 cockroach muscles using the same method of SDS–polyacrylamide gel electrophoresis[3,4] and have obtained banding patterns very similar to his for the muscles he analysed (Fig. 2). Muscles 178 and 179 have a pattern which includes the bands which Denburg labels F1 and F2 (Fig. 2, pattern 1). Muscles 177d′ and 177e′ have a pattern with the F1 and F2 bands and also the bands S1–7 (Fig. 2, pattern 2). The pattern from muscles 177d and 177e does not have the F1 and F2 bands but has bands S1–7 and an additional band, S8, which is supposed to be associated with inhibitory innervation[3] (Fig. 2, pattern 3). Our gels show a number of differences from

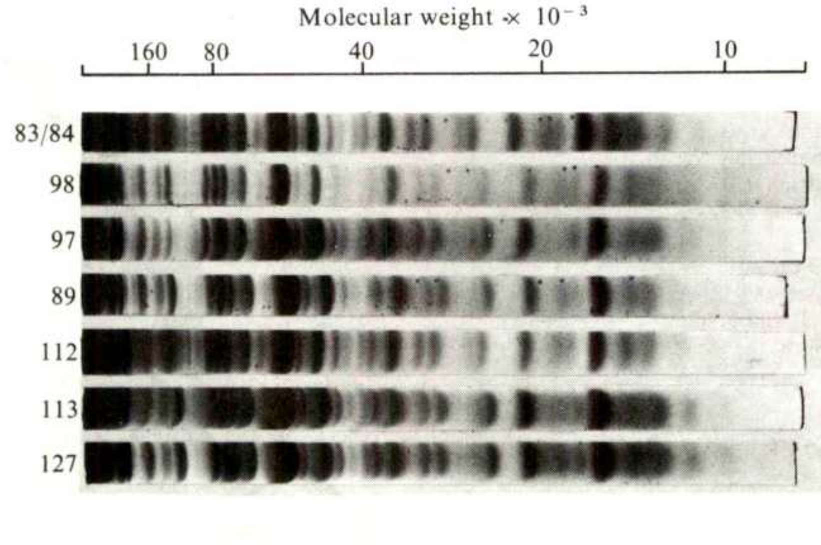

Fig. 3 SDS gels of the locust muscles shown in Fig. 1*b*, prepared in the same way as those in Fig. 2. The cells innervating these muscles are known[10-12] and are distinct, and yet the banding patterns show no distinct differences from one another. It is concluded that none of these bands can represent molecules by which ingrowing nerves could recognise specific muscles.

Denburg's, for example, an additional band labelled * (Fig. 2) but these are minor.

Analysis of other cockroach muscles (Fig. 1) shows that coxal muscles 181a, b and 182, and the thoracic muscles 174 and 176 also show pattern 1 (Fig. 2). Muscles 180 and 181c, 182c, d and 177c show pattern 2, which is also seen in the thoracic muscles 161, 163 and 167 (not illustrated). Muscle 177a shows the pattern 3 banding (Fig. 2). These results show that the characteristic patterns are not confined to those muscles innervated by cells 28 and 52 and argues against their representing specific recognition molecules. Intracellular microelectrode recording from the metathoracic coxal muscles[6,7] and from the homologous muscles in the mesothoracic segment[8] tends to support the alternative hypothesis that the patterns are related to 'fast' and 'slow' innervation (see Table 1). Pattern 1 muscles 181a, b are the homologues of the mesothoracic muscles 139a, b which Usherwood[8] has shown have only 'fast' innervation. The innervation of 174 and 176 is unknown but it is predicted that these have only 'fast' innervation. Pattern 2 muscles 182c, d have 'mixed' innervation[7], while 180 and 181c are homologous with 138 and 139c which also have 'mixed' innervation[8]. The innervation of thoracic muscles 161, 163 and 167 is unknown but it is predicted that these also have 'mixed' innervation. There are two anomalous results. Muscle 177a is a pattern 3 muscle and 177c is a pattern 2 muscle yet the mesothoracic homologues 135a and 135c have only 'fast' innervation[8]. This may reflect a true difference between the meso- and metathoracic segments, but it is more likely that the discrepancy arises from the difficulty of identifying unequivocally the subdivisions of these muscles from the accounts given in the literature[5,9].

Further evidence against Denburg's interpretation is provided by analysis of a group of flight muscles in the locust (Fig. 1*b*) in which the innervation is well known both physiologically and by intracellular filling of the motoneurones[10-12]. Muscles 97, 113 and 127 each have one 'fast' motoneurone, muscles 83/84, 89 and 98 are innervated by the two 'fast' neurones each, and 112 by five 'fast' neurones[10-12]. The banding patterns from extracts of these muscles are different from those in the cockroach, but almost identical with each other (Fig. 3). Each of these muscles has a distinct role in flight behaviour, and coordination would be impossible if a muscle were wrongly innervated. Even so, depressor muscles 97 and 127 which are immediately adjacent to elevator muscles 83/84 and 113 (Fig. 1*b*) have the same banding pattern. The bands detected by electrophoresis cannot, therefore, represent molecules by which an ingrowing nerve recognises its correct muscle. It is concluded that the bands observed are characteristic of 'fast' innervated locust muscle.

Table 1 Summary of analysis of cockroach muscles (column 1), and their pattern types as determined by polyacrylamide gel electrophoresis (column 3)

Metathoracic muscle number	Equivalent mesothoracic muscle number (where relevant)	Pattern type	Type of innervation
178	—	1	Fast[6]
179	—	1	Fast[6]
181a, b	139a, b	1	Fast[8]
174	—	1	Unknown
176	—	1	Unknown
177d′, e′	—	2	Mixed[6]
180+181c	138+139c	2	Mixed[8]
182c, d	—	2	Mixed[7]
161	—	2	Unknown
163	—	2	Unknown
167	—	2	Unknown
177c*	135c	2	Fast[8]*
177d, e	—	3	Slow
177a*	135a	3	Fast[8]*

The equivalent muscles in the mesothoracic segment for which the innervation is known from microelectrode studies[8] are given in column 2. The type of innervation deduced from these and other microelectrode studies[6,7] is given in column 4. The two anomalous results are shown by *. These may be caused by difficulty of identifying unequivocally these subdivisions of muscle 177.

We suggest that the distinctive bands detected by gel electrophoresis of whole muscle are indicative of the physiological type of the muscle rather than representing specific recognition molecules. They could be associated with either the receptor proteins or contractile mechanisms and this is worth investigating further. An immediate benefit of the results is that they demonstrate that the method may be useful for identifying the type of innervation of individual muscles.

We thank Dr Denburg for stimulating correspondence over our differences in interpretation of these results and for pointing out a discrepancy in our original interpretation of the gels for muscle 182c, d. We thank also Dr K. J. Indge and Mr G. Perry for help with electrophoresis and Drs J. S. Altman and M. D. Houslay for reading the manuscript.

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Increased membrane fluidity precedes fusion of muscle cells

Embryonic skeletal muscle cell cultures are highly suitable for demonstrating the dynamic role of the plasma membrane in cellular differentiation. A primary event in the overt development of muscle in culture is fusion of the plasma membranes of neighbouring mononucleated myoblasts to form multinucleated myotubes[1,2]. Previous studies of muscle fusion have focused on ultrastructural[3] and electrophysiological[4] aspects, the requirement for Ca^{2+} (ref. 5), and the importance of fusion for the subsequent biosynthesis of muscle-specific proteins[4]. But, understanding of this process has been hampered by the absence of reliable markers for the initiation of myoblast fusion[4]. In addition, the importance of the lipid properties of the cell membrane in regulation of the onset of muscle fusion has not been unambiguously demonstrated in spite of the well-established role of lipids in other cases of membrane fusion[6]. We examined the possibility that fusion of myoblasts is regulated by changes in the lipid fluidity of the cell membrane, as measured by the rotational diffusion of a fluorescent probe. We report here that, as a primary step in myogenesis in culture, mononucleated myoblasts undergo a sharp decrease in membrane microviscosity, and shortly afterwards fuse rapidly to form multinucleated myotubes. The microviscosity remains at a minimal value during the period of rapid cell fusion. The subsequent post-fusional differentiation of the muscle cell is accompanied by the regeneration of membrane rigidity.

Primary cultures of embryonic chick breast muscle cells were prepared[7] and plated at initial densities of $1.5–2 \times 10^6$ cells per 60-mm culture dish. Cultures were grown in Dulbecco's modified Eagle's medium[8] (88%, v/v) supplemented with horse serum (Gibco, 10%, v/v) and chick embryo extract (2%, v/v). The extent of fusion was expressed as the fraction of nuclei located in multinucleated cells[9].

Microviscosity was determined by monitoring the fluorescence polarisation of the lipid probe 1,6-diphenyl-1,3,5-hexatriene (DPH) as previously described[10,11]. Fluorescence labelling of cultured muscle was carried out by incubating muscle cell suspensions in Dulbecco's phosphate-buffered saline (PBS) (ref. 12) containing 1 μM DPH (ref. 11) for 15 min at 37 °C. Labelled cells were washed and suspended in PBS at a concentration of 0.2–0.4 mg cell protein per ml. As shown by fluorescence microscopy, the DPH-induced fluorescence was confined to the muscle cell periphery.

In the culture conditions used in this study, the differentiation of muscle cells is marked by a high degree of synchrony. Within an interval of approximately 1 d, synchronously differentiating cultures of embryonic chick myoblasts withdraw from the mitotic cycle, undergo rapid fusion, and initiate the accumulation of contractile proteins[9] and specialised membrane components such as the acetylcholine receptor and acetylcholinesterase[13]. As shown in Fig. 1, fusion of myoblasts was initiated during the second day in culture. The proportion of fused cells increased sharply and most nuclei were located in multinucleated myotubes within several hours. During the initial day in culture and before fusion, myoblasts underwent a steep decrease in microviscosity (Fig. 1). The microviscosity reached minimal levels concomitantly with onset of myoblast fusion and remained unchanged during the main period of fusion. Shortly after the degree of fusion had reached a maximum, an increment in microviscosity could be detected. Post-fusional differentiation of myotubes was accompanied by a continuous increase in microviscosity (see Fig. 1) coinciding with the developmental changes in biochemical[13] and electrophysiological[14] properties of the muscle cell membrane. This rise was maintained during the second week of maturation in culture to reach values higher than the initial microviscosity measured at the time of plating. Thus the pre-fusional decrease in microviscosity is a transitory event which is reversed in the course of subsequent differentiation of muscle in culture.

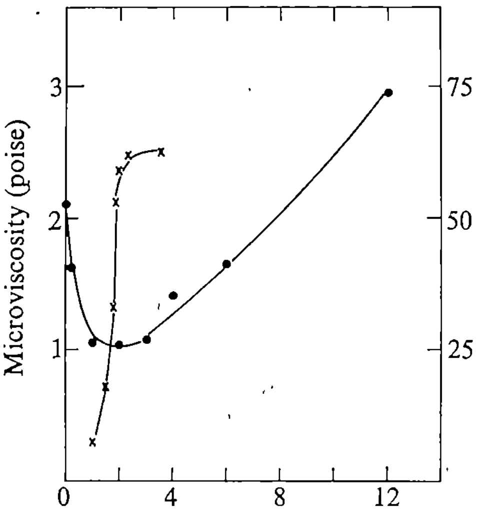


Fig. 1 Kinetics of fusion and change in microviscosity during differentiation of embryonic chick skeletal muscle in culture. Fusion (×) is expressed as the % of nuclei found in multinucleated cells. Microviscosity (●) was measured at 25 °C as before[10,11] with muscle cells labelled with DPH as follows. Cultures were rinsed with PBS and cells were suspended by 5 min exposure to 0.2% trypsin solution (Gibco). After a rinse with 5 ml PBS containing 0.25 mg Lima bean trypsin inhibitor (Sigma), the cells were resuspended in the labelling dispersion of 1 μM DPH in PBS and incubated at 37 °C for 15 min. The cells were collected by centrifugation at 2,000 r.p.m. for 5 min and resuspended in PBS. As an alternative procedure, the muscle cell monolayer was fluorescently labelled on the culture dish by exposure to 5 ml of the labelling dispersion for 15 min at 37 °C. The cells were rinsed with buffer, removed from the dishes as described above, rinsed and resuspended in PBS. Similar results were obtained with both labelling procedures. For convenience, cells were routinely labelled in suspension and measurements were performed with 0.2–0.4 mg cell protein per ml PBS.

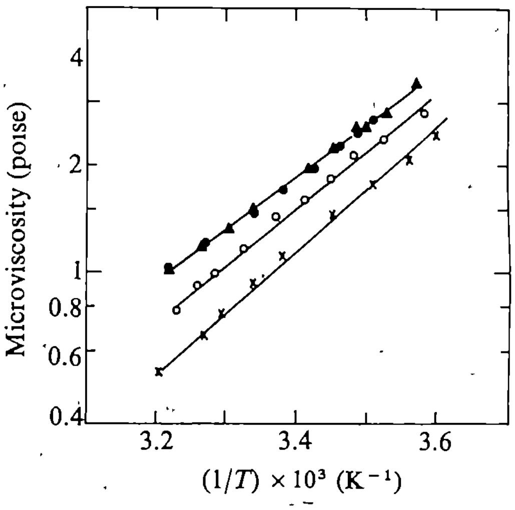
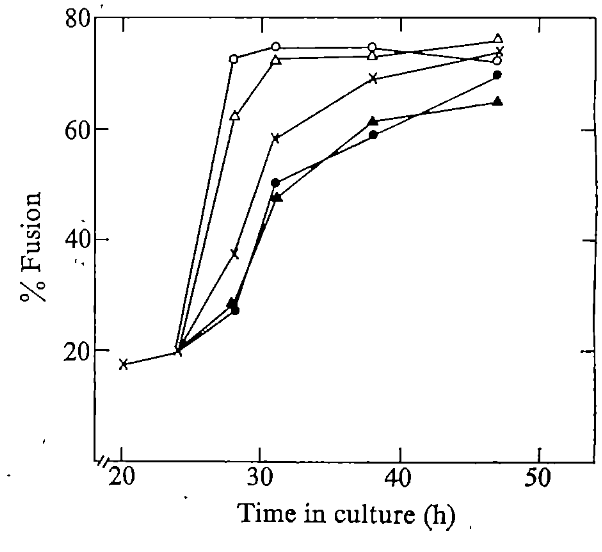


Fig. 2 Temperature dependence of microviscosity of muscle cultures at various stages of development; ●, 5 h; ×, 48 h; ○, 96 h; ▲, 144 h after plating.

Figure 2 shows the dependence on temperature of microviscosity in muscle cells at various stages of maturation. As shown, the relation between microviscosity (η) and the absolute temperature (T) fits the expression $\eta = A\,\exp(\Delta E/RT)$ which eliminates the possibility of lipid phase transitions in the muscle cell membrane both before and after fusion[15]. As calculated from the plots of log η against $1/T$ (Fig. 2), the flow activation energy (ΔE) for all cultures tested, falls in the range of 6.5–8.0 kcal mol^{-1}, a value characteristic of cell membranes[11].

The marked increase in membrane fluidity observed before the fusion of myoblasts is consistent with the suggestion that high lipid fluidity is essential for fusion[16].

Fig. 3 Effects on kinetics of fusion of exposure of myoblasts to fatty acids. Fusion was scored after addition of 2×10^{-5} M stearic (●), elaidic (▲), oleic (△) or linoleic (○) acids to replicate culture dishes 24 h after plating and compared with control cultures (×). The extent of fusion was expressed as the fraction of nuclei located in multinucleated cells[9].



This notion is supported by the reported inhibitory effects of cholesterol and reduced temperature on myoblast fusion[17]. Moreover, we have observed that exposure of myoblasts to fatty acids that increase membrane microviscosity (stearic or elaidic acids, 2×10^{-5} M) (ref. 18) retarded fusion (Fig. 3). Conversely, treatment of cells with fatty acids that decrease membrane microviscosity (oleic or linoleic acids, 2×10^{-5} M) (ref. 18) facilitated fusion. These findings suggest the importance of cell membrane lipid properties in the regulation of muscle cell fusion and indicate that the sharp increase observed in myoblast membrane fluidity is a reliable marker for the initiation of fusion. The transient fluidisation of the myoblast membrane which allows fusion could be regulated by developmental changes in lipid composition such as an overall increase in phospholipid content or increase in the degree of unsaturation of acyl chains.

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Evidence for multispecificity of antibody molecules

ONE of the salient questions in immunology concerns the means by which the immune system is capable of recognising an apparently limitless variety of antigenic determinants, including many that presumably did not exist during its evolution. Three major hypotheses have been put forward to explain the diversity of the immune response: a stringent germ line hypothesis, a recombinational germ line hypothesis and a somatic mutation hypothesis (see ref. 1 for discussion). If the assumption is made that an antibody-combining site has a single antigenic specificity, the first of the above hypotheses postulates about 1,000 variable genes and 1,000 constant genes to code for about 10^6 antigenic determinants. The second and, especially, the third hypotheses require the presence of many fewer genes in the germ line. Even fewer genes would be required if antibody-combining sites were multispecific. Evidence has been put forward to support the concept of multispecificity[2]. Moreover, the ability of single myeloma globulins to bind structurally diverse molecules[3] and the multispecific character of a Bence-Jones[4] dimer can be taken as additional supportive evidence. In an earlier paper[5], we attempted to investigate the question of multispecificity by studying the binding specificities of a population of antibodies of limited heterogeneity generated by immunisation with a conjugate of adenosine-5′-phosphate and gramicidin S: $(AMP)_2$–gramicidin S (ref. 6). Immunised rabbits produced predominantly three or four globulin proteins specific for AMP. Binding studies on the sera of one of the rabbits, in which there were three major AMP-specific globulins, yielded data that could be interpreted as evidence for multispecificity of antibody-combining sites. We have now succeeded in isolating three homogeneous anti-AMP antibodies from one of the rabbits immunised with $(AMP)_2$–gramicidin S and have studied the ability of each globulin to bind AMP and other ligands. The isoelectric pattern of the specifically purified[7] population of antibodies obtained from the serum of rabbit 449 (ref. 5) showed three major bands. The three bands were separated and isolated by preparative isoelectric focusing in polyacrylamide by an adaption of the procedure of Wrigley[8], and were shown to be homogeneous by analytical isoelectric focusing. They were designated fractions A, B and C.

Table 1 Inhibition of reaction between antibody and AdGase by haptens (relative concentration for 50% inhibition)*

Inhibitors	Fraction A	Fraction B	Fraction C
AMP	1 (8 nmol)†	1 (8 nmol)	1 (10 nmol)
DNP–glycine	75	8	100
DNP–lysine	75	10	>1,000
Menadione	2	16	>1,000
Hydralazine	825	26	>1,000
Caffeine	6	50	4
GMP	575	83	>1,000
Sodium barbital	>1,000	>1,000	>1,000
Procaineamide–HCl	>1,000	>1,000	>1,000
CMP	>1,000	>1,000	>1,000
UMP	>1,000	>1,000	>1,000
TMP	>1,000	>1,000	>1,000

*Twenty-five microlitres of a 1:50 dilution of each purified antibody (final concentration about 5 μg ml^{-1}) in 0.85% NaCl–1% rabbit serum albumin were incubated with 25 μl inhibitor (1 nmol to 10 μmol) in 0.85% NaCl for 2 h at 25 °C. To this mixture was added 25 μl Ad Gase (ref. 9) (originally 10 mg ml^{-1}) diluted 1:10,000 in 0.1 M phosphate buffer, *p*H 7.0. After incubation at 4 °C overnight, 25 μl normal rabbit globulin (2 mg ml^{-1}) and 200 μl of sheep antirabbit globulin (25 mg ml^{-1}) were added and the mixture was incubated for another 2 h at 4 °C. Seven-tenths of a milliliter of 0.01 M Tris-HCl buffer, *p*H 7.5, containing 1% RSA, 0.01 M $MgCl_2$, 0.01 M NaCl and 0.01 M mercaptoethanol were added to each tube. The tubes were then centrifuged at 3,700*g* for 15 min at room temperature. One-hundred microlitres of the supernatant were added to a test tube containing the following mixture: 1.0 ml 0.3 M phosphate buffer, *p*H 7.0, containing 0.003 M $MgCl_2$, 0.6 ml distilled water, 0.3 ml 1 M mercaptoethanol and 1.0 ml solution of fluorogenic substrate, 4-methylumbelliferyl-β-D-galactopyranoside (Nortok Associates, Lexington, Massachusetts) taken from a solution containing 14 mg per 100 ml in 0.3 M phosphate buffer, *p*H 7.0. The mixture was incubated at 25 °C for 18 h. Then, 2 ml 0.5 M glycine–NaOH buffer, *p*H 10, were added to the assay mixture to terminate the reaction, and the fluorescence was measured in a Perkin Elmer Model MPF-2A Fluorescence Spectrophotometer (excitation 360 nm, emission 440 nm). Normal rabbit globulin (1:50 dilution) was used in place of antibody as a control.

†Figures in parenthesis indicate amount of AMP to give 50% inhibition.

The specificity of each antibody was examined by an enzyme immunoassay procedure, in which binding of adenosine-β-galactosidase[9] (AdGase) was determined in the presence and absence of competing ligands. The results are given in Table 1. For all three fractions, AMP inhibited binding of Ad Gase best, but CMP, UMP, TMP and, except for fraction B, GMP were relatively poor inhibitors. On the other hand, fraction A cross reacted significantly with menadione, caffeine, DNP–glycine and DNP–lysine. Fraction B was the least discriminating of the antibodies, significant cross reactions being observed with six ligands. Fraction C, on the other hand, cross reacted well with only one of the ligands chosen for our studies, namely caffeine.

The results given in Table 1 were confirmed for fraction A by a haemolytic assay with sheep red blood cells coated with adenosine (Table 2). Inhibition of haemolysis was best with AMP followed in order of decreasing effectiveness by menadione, caffeine and DNP–glycine. GMP, hydralazine, sodium barbital and procaineamide were very poor inhibitors. These results closely approximate those obtained in the enzymeimmunoassay (Table 1).

To estblish with greater certainty that cross reactions were indeed occurring at the combining sites of the antibodies, a study was made of direct binding of ligand-coated red cells and subsequent lysis by complement. The results (Table 3) show that fractions A and B caused lysis of AMP-, DNP- and menadione-coated cells. Fraction C did not cause lysis of menadione-coated cells. These results are consistent with the findings presented in Tables 1 and 2.

Table 2 Haemolytic inhibition assays with fraction A and sheep erythrocytes coated with adenosine*

Inhibitors	Inhibition Titre†
AMP	1 nmol
Menadione	1 nmol
Caffeine	5 nmol
DNP–glycine	50 nmol
GMP	100 nmol
Hydralazine	100 nmol
Sodium barbital	100 nmol
Procaineamide–HCl	1 μmol

*Adenosine-coated erythrocytes were prepared as follows. Adenosine (20 mg) was dissolved in 1 ml 0.1 M $NaIO_4$ and allowed to stand for 20 min in the dark. One drop of ethylene glycol was then added. A 5% sheep erythrocyte suspension (1 ml) in 0.01 M phosphate-0.85% NaCl, *p*H 7.5 (PBS), was added to the periodate solution and the *p*H was adjusted to 8 with 0.1 M NaOH. Thirty minutes later, 83 mg $Na(H_3BCN)$, freshly dissolved in 1.0 ml PBS was added and the suspension was allowed to stand for another 15 min. The adenosine-coated erythrocytes were then washed twice in PBS and made up as a 0.75% (v/v) suspension in PBS. The passive haemolytic titre of fraction A was determined by microtitration; PBS, *p*H 7.0, was used as diluent. Aliquots (25 μl) of serially twofold-diluted antibody samples, 25 μl 0.75% (v/v) suspension of adenosine-coated sheep erythrocytes, 5 μl sheep anti-rabbit globulin (diluted 1:100) and 10 μl guinea pig complement (Pel-Freez Biologicals) (diluted 1:10 with PBS) were pipetted, in that sequence, into test tubes. After 1 h at 37 °C, the haemolytic endpoint was found to be at 1:16 dilution of antibody. For the haemolytic inhibition studies, 5 μl various inhibitors (100 pmol to 1 μmol) were added to 25 μl fraction A diluted 1:8. After 1 h at room temperature, 25 μl 0.75% adenosine-coated erythrocyte suspension, 5 μl sheep anti-rabbit globulin (diluted 1:100 in PBS) and 10 μl guinea pig complement (diluted 1:10 in PBS) were added. Haemolysis was allowed to proceed for 1 h at 37 °C and the titre determined.

†Lowest quantity of inhibitor for which there is still inhibition of haemolysis.

Cross specificity could also be demonstrated at the surface of lymphocytes. Peripheral lymphocytes were obtained[10] from rabbit 449. Their ability to bind AdGase in the presence of various ligands was determined by a modification of a published fluorometric assay[9,10]. The results (Table 4) show that at the single concentration measured, AMP, menadione and hydralazine were essentially identical in their ability to displace AdGase from the population of lymphocytes capable of binding the latter. With respect to the other ligands, the activities were as follows: barbital > caffeine > GMP > DNP–glycine. Procaineamide and UMP were essentially inactive. The results show that receptor sites on peripheral lymphocytes are also multispecific. Generally, ligands that competed well were the same ones that did so in the experiments with circulating antibody. Exact comparisons are not valid, however, since in the lymphocyte assay one is also examining the specificities of cells that were not stimulated to produce anti-AMP antibodies by $(AMP)_2$–gramicidin S. We know from our previous studies[5] that A-ovalbumin stimulated at least a hundredfold more clones than did $(AMP)_2$–gramicidin S. These additional cells should also bind AdGase and have different spectra of cross-reactions.

Our data support the notion[2] that combining sites of antibodies are multispecific. Thus, they share the property of other ligand-binding proteins (such as enzymes) of being able to bind more than a single ligand. Like enzymes, they can bind ligands of diverse structure, and, again, as with enzymes, effective ligands need have no obvious resemblance to the primary 'specific' ligand (see, for example, ref. 11). Antibody-combining sites can be large enough to accomodate five or six sugar residues[12]. The contact surfaces, therefore, are complex. One would not expect to be able to predict the types of different structures that can be accommodated. Indeed, our results show that predictions are not feasible. Our anti-AMP antibodies do not bind GMP, UMP or TMP well. Yet, they bind such molecules as menadione and hydralazine. With respect to the former, we have carried out preliminary studies to determine the types of forces involved in its binding to fractions A and B. Surprisingly, its ability to bind decreased markedly with temperature indicating that hydrophobic forces are not predominant[13]. AMP, on the other hand, bound equally as well at 0 °C and at 37 °C, suggesting that hydrogen bonds, electrostatic forces and hydrophobic forces might all be involved in its binding to specific antibody.

Table 3 Haemolytic assys with hapten-coated sheep erythrocytes*

	% Lysis of coated sheep erythrocytes†			
	.Anti-AMP			
Hapten	Fraction A	Fraction B	Fraction C	Anti-A ovalbumin
Adenosine	100 (1:16)‡	100 (1:8)	100 (1:8)	100 (1:640)
DNP	45	81	16	100 (1:64)
Menadione	60	24	0	100 (1:32)

*DNP-erythrocytes: 2,4-dinitrobenzene sulphonic acid (sodium salt; Eastman) in 1.0 ml PBS was added to 1.0 ml 5% suspension of sheep erythrocytes in PBS. The suspension was left at room temperature with intermittent shaking for 2 h. Excess reagent was removed by washing several times with PBS. Menadione-coated erythrocytes: A saturated solution of menadione was made up in PBS. A 5% erythrocyte suspension (1 ml) was exposed to 5 ml of the saturated solution for 30 min, followed by two washings with PBS.

†Haemolytic assay carried out as described in Table 2. Quantification of haemoglobin released from lysed erythrocytes was carried out by adding 1 ml PBS to each testtube after the second incubation period. The supernatant was recovered by centrifugation at 150*g* for 5 min at 4 °C and haemoglobin was determined spectrophotometrically at 413 nm. Haemoglobin released by cells lysed by distilled water represented 100% lysis.

‡Numbers in parenthesis denote highest dilution of antibody that caused lysis; otherwise, antibody was undiluted and was at concentration of about 200 μg ml^{-1}. Uncoated sheep erythrocytes + antibody and coated erythrocytes with pre-immune sera showed no lysis.

Although we think it likely, our data cannot be used to show that different subsites of the combining site are involved in the binding of various ligands. We would like to establish this with certainty by attempting to isolate sufficient antibody protein to attempt

Table 4 Inhibition of binding of AdGase to lymphocyte surface receptors by various inhibitors

Inhibitors (20 nmol)	% Inibition†
AMP	28 ± 3
Menadione	31 ± 3
Hydralazine	28 ± 3
Sodium barbital	20 ± 2
Caffeine	12 ± 1
GMP	7 ± 1
DNP–glycine	2 ± 1
Procaineamide	0
UMP	0

The assay procedure was a modification of one described previously[10]. Peripheral blood lymphocytes (2×10^6 in 50 μl PBS) were incubated with 20 nmol ligand in 25 μl PBS at 4 °C. After 90 min, 50 μl of a 1:1000 dilution of AdGase were added and incubation was continued at 4 °C for another 3 h. PBS (2 ml) was added to each sample and the cells were collected on 25-mm diameter Whatman No. 1 filters placed in a Millipore manifold. A 1:1000 dilution of AdGase (50 μl), without cells, was also filtered through the paper to serve as a blank. All filters were then washed with 20 ml PBS and placed in test tubes containing the assay solution described previously (footnote to Table 1) and incubated for 30 min at 25 °C in a shaking water bath. All subsequent procedure described previously[10].

†Given as mean ± s.d. mean of four experiments done in duplicate.

crystallisation and X-ray crystallographic examination. This is not necessarily a trivial point since it would definitely rule out the possibility that all competing ligands resemble each other sufficiently to explain the crossreactions. For example, Michaelides and Eisen[14] used this argument to explain cross reaction between menadione and DNP in myeloma proteins. This is a very difficult argument to dispose of, since interactions between ligands and binding proteins take place mainly by way of functional groups and/or hydrophobic surfaces. There are only a limited number of the former, and very many ligands have hydrophobic areas. Thus, we can always find structural resemblances, particularly with small ligands. Each of our three purified AMP-binding proteins, however, has its own cross specificities, a finding that would not have been expected if there were thermodynamically important structural similarites among the ligands. The finding that each of the anti-AMP proteins bound a different spectrum of other ligands is in agreement with the proposal of Richards *et al.*[2] that the high specificity of the immune response lies not only in the properties of a single antibody molecule but is due also to the production of a heterogeneous population of antibody molecules all sharing specificity for AMP (as we found with A-ovalbumin as an immunogen)[5] but with each member having its own spectrum of crossspecificities.

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Internal motions of antibody molecules

ANTIBODY molecules of the IgG class are composed of three covalently linked regions[1,2]. Two of these (designated Fab) are identical and bind antigen; the third (Fc) has been identified as the site of antibody effector functions (for example, complement fixation), which are activated by antigen binding[3]. There is evidence that the binding of antigen to the Fab region induces an allosteric transition in IgG and that this transition may be a step in the effector activation process[4]. The findings of Pecht *et al.*[5] indicate that for complement fixation both Fab regions must bind antigen, even though binding to one induces some conformational change in Fc. Binding of hapten does not lead to complement fixation in general, but some exceptions to this rule are known[6]. The interchain disulphide bridges must be intact for Fab ligand binding to have a productive effect on the Fc region[7,8]. Both the Fab and Fc regions have a domain structure; each consists of a pair of compact lobes covalently linked by strands of polypeptide chains[9]. A schematic diagram of the connectivity of the IgG lobes is given by Padlan[10]. One of the fundamental questions involved in antibody function is the nature of the mechanism by which binding of antigen to the Fab regions affects the Fc region. Huber *et al.* have recently outlined a possible allosteric model for the activation step on the basis of X-ray and other data[9]. In this note we use simple diffusion arguments to estimate the characteristic times associated with the Huber proposal. Since the analysis deals only with the relative motion of the IgG domains, it is possible that slower intradomain structural changes are also involved in the activation process; if so, the estimated characteristic times would be lower bounds for the activation time. The role of conformational transitions in effector function activation is still in dispute[11], but we note that the diffusional model may be useful for analysing other dynamical phenomena in immunoglobulins, such as their fluorescence depolarisation behaviour[12–14].

Huber *et al.*[9] assume that the lobes have relatively little interaction with each other in the unliganded IgG. Thus, neighbouring lobes are free to rotate relative to each other over certain angular ranges. Inspection of the Fab regions in the IgG structure suggests that the V_L–V_H dimer (the antigen-binding lobe) can rotate about an axis passing through the 'switch peptides' which connect it to the C_L–C_H1 dimer (the second lobe in Fab); this motion has been termed 'elbow bending'. The Fab region itself can rotate relative to the Fc region by a 'hinge bending' deformation of the peptide which links the C_L–C_H1 dimer to C_H2–C_H2, the first lobe of the Fc region. Although the 'elbow' and 'hinge' bending motions are the only ones that concern us here, it should be noted that there may also be a flexible link between the two Fc lobes.

The binding of antigen is postulated to trigger the successive 'locking' of some of these bending motions[9]. In particular, it is suggested that antigen binding induces a conformational transition in the V_L–V_H dimer that causes it to interact more strongly with the C_L–C_H1 dimer. This interaction is assumed to induce a conformational transition in the C_L–C_H1 dimer that alters its affinity for the C_H2–C_H2 dimer. The resulting coupling between the Fab and Fc regions is presumed to lead to the activation of Fc.

The relative displacements of the various domains in going from the open (unliganded) to the locked (liganded) structure can be characterised as bending motions. We assume in what follows that these bending motions can be described by a one-dimensional diffusion equation. The justification for this assumption comes from a recent theoretical study of the hinge bending mode in lysozyme[15]. It was found there that in spite of the forces due to the interactions between the two lobes (covalent plus non-bonded), their relative motion is diffusive in character; that is, the hydrodynamic frictional forces dominate the motion. Frictional effects are likely to be even more important in the relative motion of the IgG lobes, which are larger than those in lysozyme and apparently interact more weakly.

The one-dimensional diffusion equation is

$$\frac{\partial p}{\partial t} = D\frac{\partial^2 p}{\partial \theta^2}$$

where $p(\theta, t)$ is the probability that the elbow or hinge angle is θ at time t and D is the angular diffusion constant appropriate to the particular motion. The boundary conditions are $p(0, t) = 0$, representing locking at the angle $\theta = 0$, and $\partial p/\partial\theta = 0$ at $\theta = \theta_r$, corresponding to reversal of the bending motion at the maximum excursion angle, θ_r. If all bending angles between 0 and θ_r are equally populated initially, the average time required to reach $\theta = 0$ is $\tau = \theta_r^2/3D$ (ref. 16); in the limiting case that all molecules have $\theta = \theta_r$ initially, the average time becomes $\tau = \theta_r^2/2D$.

To obtain approximate values for the angular diffusion constants, we model the domains involved by spheres of radius a and treat the bending motions as relative rotations about an axis located a distance r from the sphere centres. The effect of the fluid environment is represented by using the Stokes law for the frictional coefficient of the spheres[17]; the correction factors for relative motion and hydrodynamic interaction approximately cancel for rough spheres which are close together[18]. The calculated diffusion constants in water at 300 K ($\eta = 1\times10^{-2}$ P) are $D_e \approx 2.8\times10^7$ s^{-1} for the elbow bending ($a \approx r \approx 20$ Å) and $D_h \approx 3.4\times10^6$ s^{-1} for the hinge bending ($a \approx r \approx 40$ Å).

To complete the estimate of the average times required for locking, we need to know the ranges of angular excursion, θ_r. If we use the angular differences between the 'minimum disorder' unliganded IgG structure and the hypothetical liganded structure proposed by Huber *et al.*[9], they are $\theta_r \approx 0.7$ rad (40°) for the elbow, and $\theta_r \approx 0.5$ rad (30°) for the hinge. The resulting average time for elbow locking is $\tau_e \approx 6\times10^{-9}$ s and that for hinge locking is $\tau_h \approx 3\times10^{-8}$ s.

It is possible to test the diffusion constant values by using them to analyse fluorescence depolarisation results for antibody molecules[12,13]. We consider a large molecule with isotropic rotational diffusion constant D_{IgG} to which a labelled internal rotor with uniaxial rotational diffusion constant D_h is attached. If the angular range of internal rotation were unrestricted, the fluorescence anisotropy of the label would be a sum of exponentials, each decaying with a certain characteristic time; the characteristic time for the fastest decaying term would be $\phi_h = 1/(6D_{IgG}+4D_h)$ (ref. 19). This expression should also provide a useful estimate of the decay time due to Fab motion in IgG, although the range of internal motion is somewhat restricted in this case. Taking $6D_{IgG} = 1/(1.7\times10^{-7}$ s) from the work of Yguerabide *et al.*[12] yields $\varphi_h = 5.1\times10^{-8}$ s, which is near the shortest decay times measured for antibodies labelled with fluorescent haptens (3.3×10^{-8} s) (ref. 12). Thus, these results indicate either that Fab–Fc locking is somewhat less rigid in liganded antibody than supposed by Huber *et al.* or that hapten is less effective than antigen in inducing the Fab–Fc locking; circular polarised fluorescence studies support the latter possibility[5]. It is worth noting that the shortest decay times measured in similar studies using nonspecific, covalently bound labels are somewhat smaller (10^{-8} s); (ref. 13) this corresponds to the estimated shortest decay time for a labelled V_L–V_H dimer of a completely open IgG: $\varphi_e \approx 1/(6D_{IgG}+4D_h+4D_e) \approx 8\times10^{-9}$ s. This suggests that it is not necessary to invoke independent rotation of the fluorophore to explain the short relaxation time measured by Wahl[13]. The above results, taken together, lead to the interpretation that specific binding of hapten may reduce motion about the elbow without a comparable effect on motion about the hinge, while nonspecific attachment of labels has little effect on either motion. Thus, the fluorescence depolarisation studies are consistent with a hydrodynamic model of IgG internal motions.

A number of possible refinements of the model calculations should be considered. Although the times involved in the relaxation phenomena and the locking reaction are not expected to be very sensitive to the detailed form of the elbow and hinge bending potentials (which we have assumed to be constant in the region $0 \leqslant \theta \leqslant \theta_r$ and infinite outside), the locking times do depend quadratically on the allowed ranges of motion. In particular, the hinge bending motion could involve a larger angular range in the unliganded antibody than assumed above. This would lead to a longer average locking time; a reasonable upper estimate for θ_r ($\theta_r = 90°$) gives $\tau_h \approx 2.5\times10^{-7}$ s. Another factor to be considered is that the diffusion constants D_e and D_h would be decreased by the binding of large antigens to the IgG. If the bending motions were to depart somewhat from the assumed one-dimensional rotational character, the conclusions of this work would not be altered significantly. If the probability of locking were less than unity at $\theta = 0$, the required correction factor could be introduced by using a partially absorbing barrier[20]. Finally, the detailed character of the bending potentials and their role in the bending motion and the locking reaction are of considerable interest.

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Are late replicating regions in polytene chromosomes of *Drosophila* enriched by repeated nucleotide sequences?

THE ordered highly coordinated pattern of replication of different bands is characteristic of salivary gland chromosomes of *Drosophila*[1,2]. During the initial stage of polytene chromosome replication labelled precursor is found over Puff/interband sections while prominent bands incorporate label later and need in general a longer time for DNA synthesis[3,4]. It is significant that regions of ectopic pairing in the chromosomes of *D. melanogaster* are of the late replicating type. We report here that late replicating DNA from *Drosophila* salivary gland cells reassociates after denaturation more rapidly than the bulk of the DNA from the same cells A high level of repeated nucleotide sequences may be involved.

Several autoradiographic investigations have shown that there is no strict correspondence between DNA content and replication time of chromosome bands[5,6]. In other words, it is not possible to explain the difference in the timing and duration of replication of regions studied based only on different DNA content. Thus there are at least two possibilities to explain the prolonged S-phase characteristic for definite regions in the genome of *Drosophila*:

(1) The macromolecular configuration of DNA packed into chromomers in different parts of the genome determines the number of initiation points and consequently the time necessary for the replication of a given band. Thus regions of prolonged replication may have fewer initiation sites in their DNA.

Table 1 Frequencies (%) of different labelling types at the end of third instar in *D. virilis* larvae

Time from the beginning of third instar (h)	70	72	74*
Total no. of nuclei studied	1,064	791	584
No. of labelled nuclei	284	164	96
Frequency of continuous replication pattern (%)	10.6	2.4	2.1
Frequency of discontinuous replication pattern 89.4 (final stages of S-phase) (%)	89.4	97.6	97.9

* Larvae with dark-brown anterior spiracle stage used in this investigation.

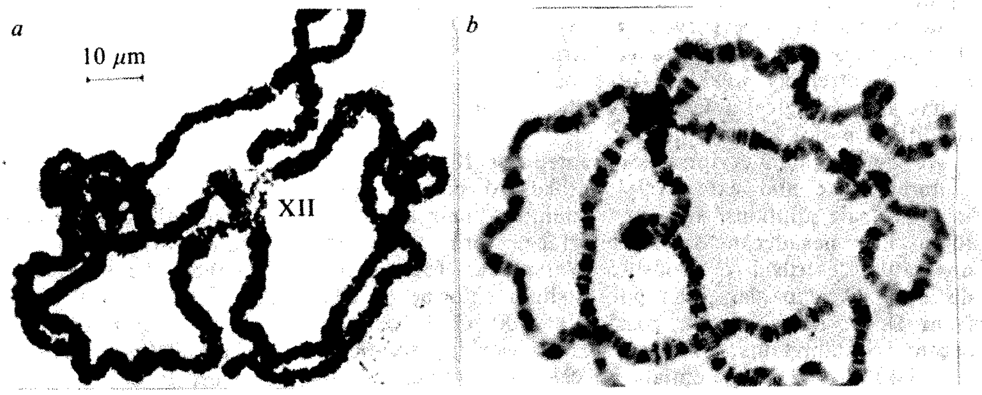

Fig. 1 *a*, Polytene chromosomes of *D. virilis* after long-term labelling; (first series) (bar represents 10 μm); *b*, polytene chromosomes of *D. virilis* after short-term labelling. Only prominent late replicating bands incorporate ^{3}H-thymidine.

(2) Late replicating regions (bands) of polytene chromosomes may be enriched by repeated nucleotide sequences. This possibility comes to mind because C-heterochromatin consisting of highly repetitive satellite DNA (sDNA) is a late replicating fraction of the genome in various organisms.

To examine the latter possibility we studied renaturation kinetics of salivary gland DNA of *D. virilis* labelled by different methods. A long-term labelling was carried out in the first series of experiments. The flies were reared from oviposition to the end of third instar larvae on a medium containing ^{3}H-thymidine (100 μCi ml^{-1}, specific activity 4.4 Ci mmol^{-1}). The salivary gland chromosome DNA of such individuals undergoes several replication cycles in the presence of labelled precursor and is thus uniformly labelled (Fig. 1*a*). In a second series of experiments (short-term labelling) the salivary glands of third instar larvae (dark-brown anterior spiracle stage) were incubated for 15 min in a drop of Ringer insect medium supplemented with ^{3}H-thymidine (100 μCi ml^{-1}, 49 Ci mmol^{-1}).

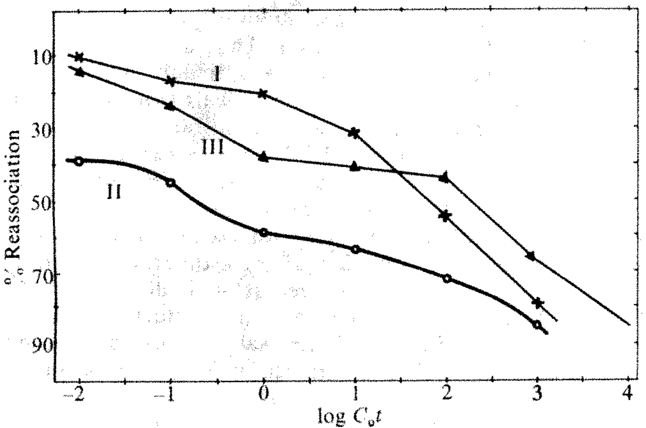

Fig. 2 Renaturation kinetics of *Drosophila virilis* salivary gland DNA labelled by different methods (I, II) and mouse DNA used as standard (III) as measured by hydroxylapatite chromatography. Samples of sheared labelled DNA were sealed in glass capillary tubes, heated to 105 °C for 10 min to denature the DNAs and then incubated at 60 °C to allow renaturation. At various times samples were removed, diluted into 1 ml of 0.12 M PB and frozen for subsequent hydroxylapatite fractionation. Single-stranded and double-stranded DNAs were eluted with ten times the bed volume of 0.12 and 0.5 PB, respectively. Renaturation was measured by ^{3}H-radioactivity of the hydroxylapatite fractions. I, *D. virilis* salivary gland DNA-long-term labelling (X). II, *D. virilis* salivary gland DNA-short-term labelling (○). III, Mouse DNA (standard) (▲).

In preliminary experiments, after short-term labelling of salivary glands from these larvae, 98% of labelled nuclei showed discontinuous labelling patterns characteristic of end phases of polytene chromosome replication (Table 1). Thus preferential labelling of DNA of late replicating regions (bands) occurred in these experiments.

Discontinuously labelled chromosomes of the final stage of the S phase are shown in Fig. 1*b*. DNA from salivary glands was isolated using the cetavlon procedure[7]. DNA was sonicated and dissolved in 0.12 M Na-phosphate buffer (PB). The average fragment size of the sonicated material is about 300–400 nucleotides.

The reassociation kinetics of DNA from both series of experiments were studied using hydroxylapatite chromatography. Comparison of C_0t curves of both DNA preparations (I, II series) shows that late replicating DNA (series II) reassociates at a faster rate than the bulk DNA (series I) and hence contains significantly more repeated sequences than the genome as a whole.

The difference observed could not be due to the late replication of sDNA which does not replicate during polytenisation or at least does not replicate as frequently as euchromatic sequences[8].

The high value of reassociation at $C_0t = 10^{-2}$ in the case of late replicating DNA (series II) can not be due to snap back of palindrom sequences. Zero-time reassociation in series II equals 7%, compared with 3.8% in series I.

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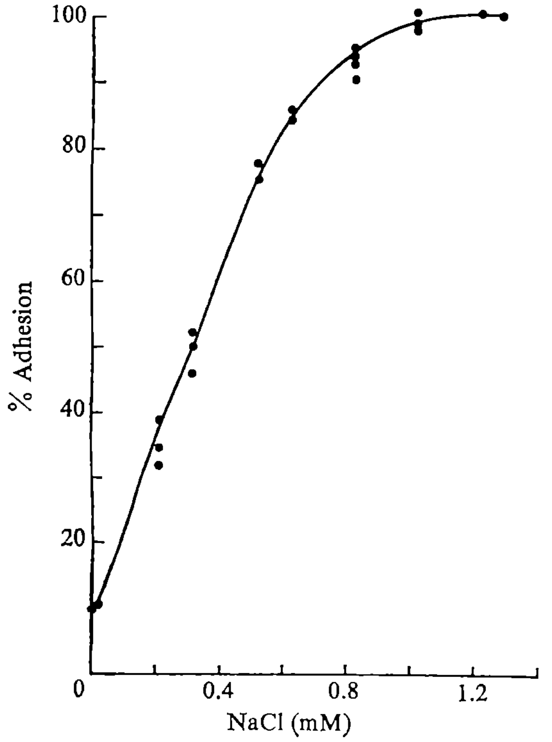
Long-range attraction between red cells and a hydrocarbon surface

WE present here experimental evidence that adhesion of red cells to an oil–water interface is mediated by an attractive force acting at a distance apparently exceeding 100 nm. Although long-range forces have recently been measured between bilayer lipid lamellae in water[1] and attraction between cells has been inferred from aggregation studies[2,3], the long-range nature of attraction in cell studies has previously been an assumption rather than a deduction from experimental evidence. Our analysis strongly implicates a long range attractive force, but this force is extremely weak at large distances and whether it has any biological role in cell adhesion in physiological conditions is not known.

We used human erythrocytes treated for 18 h in 3.3% glutaraldehyde and extensively washed, to prevent lysis in dilute salt solutions, and then examined their adhesion to a flat hexadecane–saline interface, formed in an apparatus described previously[4]. Water was four times distilled, twice in glass, and pure sodium chloride (a gift from Dr J. Mingins) was roasted at 700 °C to remove organic contaminants. The final *p*H of working solutions was 5.4±0.1. The ζ-potentials of cells and sonicated oil droplets in saline were measured in a Zeiss cytopherometer modified for low constant currents. Surface tension of method in a thermostatically controlled apparatus (±0.1 °C). Adhesion was assessed by allowing cells to sediment under gravity in salt solution onto the oil–water interface for 20 min, then inverting the whole system (oil uppermost). The number of adherent cells was recorded photographically, the total initial number counted at the interface being greater than 400 in every case. Results were unaffected by increasing settling time to 1 h before photography.

From 145-mM NaCl down to 1.0 mM, cells remained attached to the interface indefinitely after inversion; below 1.0 mM, cells fell away from the interface (see Fig. 1). In another series of experiments cells were allowed to attach to the interface either at 10 mM or at 0.4-mM NaCl, then the system was inverted and the saline was carefully exchanged for distilled water. Between 70% and 90% of the cells fell off, whereas none lost their adhesion in a control where exchange was done with 0.4-mM NaCl. Since the control excludes mechanical removal we conclude that most of the cells in dilute saline are reversibly attached. We tested for contamination of the oil–water interface by adherent cells by measuring the interfacial tension of a drop of 10 mM or 150 mM saline formed under hexadecane. When the saline contained cells which were allowed to settle onto the interface for 20 min the surface tension remained the same as for a drop lacking cells, to within our experimental accuracy of 0.1 dyn cm^{-1}. Red cells untreated with glutaraldehyde caused a rapid large fall in interfacial tension. We conclude that glutaraldehyde-treated cells did not contaminate the interface.

Fig. 1 Adhesion of red cells to hydrocarbon as a function of salt concentration.



In 0.3-mM NaCl where half the red cells fall off the interface, the interface has a potential of about −30 mV and the cells −95 mV. By modelling the biconcave red cell as a torus we calculate the electrostatic repulsion for a cell interacting *en face* with the interface (as most are seen to do). We combine the computational procedures of Alamov[5] and Brenner and Parsegian[6] using the full non-linear Poisson–Boltzmann (PB) equation in the region where potentials exceed 25 mV. The validity of the PB equation for force calculations has recently been verified in two ways[7,8]. We take[9] the smaller toroidal diameter as the red cell thickness (~1.5 μm), the length of the toroidal axis or Pappus line as 17 μm, the density in water as 0.096 g ml^{-1} and red cell volume as 85 μm^3. Such an average cell would experience a gravitational force of 8×10^{-9} dyn. This is the size of the calculated electrostatic repulsion in 0.3-mM NaCl for a cell to interface separation of about 240 nm, that is, conditions in which an average cell is about to lose its adhesion. Thus a cell sedimenting down onto the oil–water interface under gravity alone cannot approach closer than 240 nm. This conclusion is the crux of our argument. Without an attractive force, cells would balance at 240 nm and fall off on inversion Because half the population do not, an atractive force must reach out to at least 240 nm. To take account of the population spread, the calculation was done for conditions corresponding to 20% and 80% adhesion, giving estimates of the range of attractive force that can act at such distances is the van der Waals or electrodynamic charge fluctuation force.

Whether adhesion in dilute salt occurs at a finite separation as a result of a balance of long-range attraction and electrostatic repulsion or takes place at 'molecular contact' is not directly ascertainable from our measurement. Reversibility of adhesion is consistent with the action of long-range attractive forces In the absence of a mathematical expression for electrodynamic attraction between a torus (or cylinder) and a plane wall which is valid for large separations where retardation will be important, one can only say that the attractive force must decrease with increasing distance. From what is known of the strength of van der Waals forces (1) (7) (10) (11) it seems virtually impossible that an electrodynamic force could pull cells in 0.3-mM NaCl into molecular contact with the interface; a force balance at a finite separation seems inescapable. This conclusion is supported by interferometric observations (in progress) on cells adherent to the interface in 0.4-mM NaCl. Adherent cells can oscillate in a direction perpendicular to the interface while remaining separated from it by a finite gap. This behaviour is not consistent with molecular contact but is precisely what would be expected from a long-range force balance.

Detection of long-range attraction is of considerable interest from a physical viewpoint, but its biological significance remains uncertain.

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The number of turns in globular proteins

Peptide chain turns are those parts of a globular protein where the backbone is folded back upon itself. We show here that the number of turns is a linear function of the number of amino acid residues in the protein, and we compare the two contrasting models for turn formation. In a sequence-dependent model, turns are a linear function of the molecular weight, while in a shape-dependent model, they are a function of the two-thirds power of the molecular weight. But, a shape-dependent model also behaves, to a good approximation, as a linear function within the domain of interest. We suggest some consequences of this work for protein folding.

Peptide chain turns were first identified by Kuntz[1] and by Lewis *et al.*[2]. Kuntz points out that turns are important for two reasons—they constitute recognisable structural units in proteins, and they are situated at the solvent accessible surface of the molecule. Rose and Seltzer[3] devised an algorithm to identify peptide chain turns from coordinate data. This algorithm treats the polymer chain as a curve in space and computes a discrete radius of curvature for that curve. A turn corresponds to a locus where the chain direction vector is changing rapidly and the value of the radius of curvature is at a local minimum. Although turns can be shown to correspond to local minima in the radius of curvature, the correspondence is not 1:1; that is, some minima are not associated with turns, and additional analysis is developed to identify these loci. α-Carbon coordinate data are the only information required by the algorithm, and notions about hydrogen bonding at turn loci are irrelevant to the geometric nature of the procedure. In this sense, the algorithm provides an objective criterion for the recognition of turns as strictly structural components in proteins.

We have used the algorithm for turns to discover the number of turns in each of 21 proteins ranging in size from 53 to 450 amino acid residues (Table 1). A least squares analysis shows that the number of turns (T) is a linear function of the number of amino acid residues (R) as given by

$$T = 0.125R + 2.28 \qquad (1)$$

The correlation coefficient is 0.983. An equivalent correlation exists between T and the molecular weight of the protein. These data are shown graphically in Fig. 1. The analysis is especially compelling in view of the strictly structural nature of the procedure.

We have previously defined a structural segment as the three-dimensional structure assumed by a continuous sequence of linear chain neighbours bounded by consecutive chain turns[5]. Clearly identification of the turns also results in identification of the structural segments, with the number of segments always one greater than the number of turns. A structural segment, by this definition, is similar to Chothia's definition[4] of pieces of secondary structure. Thus, the number of structural segments (S) is also a linear function of the number of amino acids as given by

$$S = 0.125R + 3.28 \qquad (2)$$

Some discussion of the linear nature of the function relating turns to the number of residues is in order. In particular, a class of functions of the form

$$T = aR^n + b \qquad (3)$$

can be studied where, of course, $n = 1$ when the relationship is a linear one. A choice of $n = 2/3$, however, is appropriate to the geometric argument proposed by Kuntz[1], and it turns out that the data in Table 1 will fit such a model equally well. Indeed, given suitable coefficients a and b, models of the form given in equation (3) seem to be quite insensitive to a choice of n over the domain 0.5–1.5.

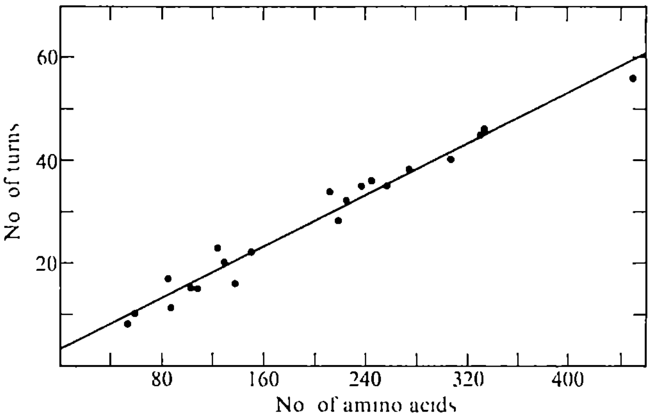

Fig. 1 A plot of no. of turns against no. of amino acids for 21 proteins. The least squares line through these points has slope 0.1245. Detailed data are given in Table 1.

To resolve the value of n, a log–log logarithmic plot of turns against number of residues was constructed. The slope of the least-squares line to these data is 0.94, much closer to linearity than to 2/3.

Two distinct models for turn formation emerge from the above discussion. A sequence-dependent model results if turns are determined by linearly local sequences of amino acids along the polypeptide chain. For a given protein with k sites for turns imbedded within the amino acid sequence, the fraction of total residues in turns would be k/R. The data in Table 1 show that k/R is reasonably constant over a large and diverse sample of globular proteins, a fact that can be attributed to the characteristic composition of globular proteins in general[10]. For this reason, the data points of Table 1 cluster well around a line with a slope that is the best-fit to the values of k/R as shown in Fig. 1. The sequence-dependent model is also consistent with the success of empirical correlations between sequence and structure[11]. The model is inherently linear, with $n = 1$ in equation (3).

In contrast, a shape-dependent model would result if turns were distributed over the globular surface of the folded protein, without particular regard for sequence. Janin[12] and Teller[13] have shown that the surface area of proteins is proportional to the molecular weight raised to the two-thirds power. Hence, a shape-dependent model with a uniform density of turns would have $n = 2/3$ in equation (3).

For the reasons given, a sequence-dependent model seems more likely, and the insensitivity of equation (3) to a choice of n implies that sequence-dependent behaviour need not be at the expense of the protein's globularity. Another way of seeing this is to consider the surface-to-volume ratio for spheroids of interest, as shown in Fig. 2. Within the size range of proteins, this ratio is nearly constant, and approximately the same for either a sphere or prolate and oblate spheroids with axial ratios of 2:1. That is, the equation

$$A/V = c \tag{4}$$

where A is the surface area, V the volume, and c a constant, is a good approximation over the domain of interest. Since the number of residues in the protein is certainly proportional to the molecular weight which is, in turn, proportional to the volume, a sequence-dependent model will vary linearly with the volume of the protein

$$\text{Turns} \propto V \tag{5}$$

while a shape-dependent model will vary linearly with the two-thirds power of the volume or the surface area

$$\text{Turns} \propto A \tag{6}$$

From equation (4), however, $A = cV$, so the shape-dependent model is also seen to vary linearly with the volume. Thus, within the size range of interest, even a shape-dependent model behaves, to a good approximation, as a linear function of the number of residues.

If geometric criteria alone determine the turns, it is unlikely that any protein of globular shape would deviate very much from the shape-dependent model; while if the local sequence determines turns, a protein of usual composition would be expected to exhibit deviations. Myoglobin is a case in point. Equation (1) predicts 21 turns for the 153 residues of myoglobin, but the algorithm for turns finds only 13. A sequence analysis of myoglobin confirms the unusual composition of this protein which gives rise to unusually long helical structural segments with few turns.

The apparent sequence-dependence in our findings was inferred from counting structural sites in the folded protein. The fact that locally determined, sequence-dependent sites are still discernible after folding can be interpreted to mean that the protein is partitioned into its structural segments and turns by local sequences of amino acids, and that these moieties tend to persist. This interpretation is compatible with our model of protein folding[6], called the LINCs and hinges model. In this model, linearly short and medium range interactions dominate early folding, causing the disordered chain to assemble first into local independently nucleated continuous segments (LINCs). LINCs are structurally persistent, separable, modular entities that are precursors to their counterparts in the folded protein, and each LINC has its own characteristic equilibrium between random coil and the conformed state. The hinges are viewed as conformationally permissive loci where the backbone changes its overall direction; it is presumed that these become chain turns.

Table 1 No. of turns measured and predicted for 21 proteins

Protein	No. of amino acids	No. of turns (measured)	No. of turns (predicted)
1 Carbonic anhydrase	258	34	34
2 Carboxypeptidase	307	39	41
3 Chymotrypsin	245	36	33
4 Concanavalin A	237	34	32
5 Cytochrome b5	87	10	13
6 Cytochrome C	103	14	15
7 Flavodoxin	138	15	19
8 Glyceraldehyde-3-phosphate dehydrogenase	334	45	44
9 Hexokinase	450	55	58
10 High potential iron protein	85	16	13
11 Lactate dehydrogenase	331	44	44
12 Lysozyme	129	19	18
13 Myogen	108	14	16
14 Papain	212	33	29
15 Phosphoglycerate mutase	218	27	29
16 Ribonuclease S	124	22	18
17 Rubredoxin	53	7	9
18 Serine protease (fragment)	225	31	30
19 Superoxide dismutase	151	21	21
20 Subtilisin	275	37	37
21 Trypsin inhibitor	58	9	10

Measured turns are found with the algorithm of Rose and Seltzer[3]; these are the data points in Fig. 1. Predicted turns are calculated from equation (1) which gives the best-fit line shown in Fig. 1. The difference between measured and predicted values is the residual for each protein data point.

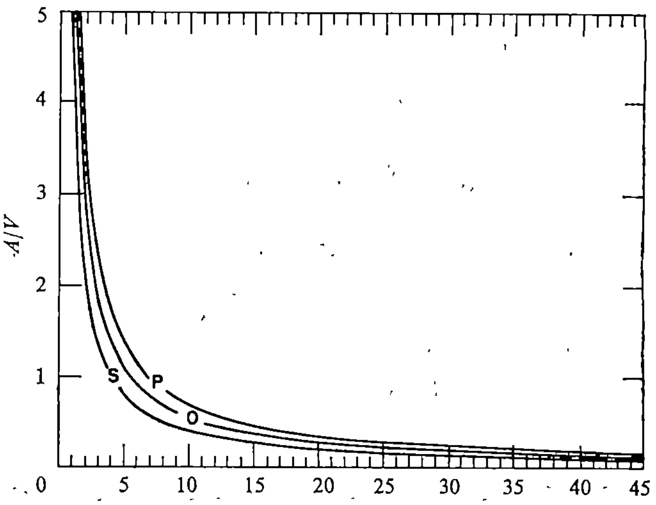

Fig. 2 A plot of the surface-to-volume ratios for a prolate ellipsoid with axial ratio 2:1 (P), an oblate ellipsoid with axial ratio 2:1 (O), and a sphere (S). The abscissa is the radius of the sphere and the length of the major axis of the other spheroids. Assuming a partial specific volume of 0.75 $\text{cm}^3\ \text{g}^{-1}$ and a perfectly spherical shape, rubredoxin would have a radius of 12 Å and LDH would have a radius of 22 Å.

In our folding model, a protein is comprised entirely of LINCs and interspersed hinges. These nascent structural elements then coalesce as a result of orientation and diffusion processes to provide a cooperative mutual stabilisation. The cooperative folding of LINCs is responsible for the emergence of larger structural domains, and, ultimately, the native structure of the protein. Not until the LINCs have coalesced into larger structures does the protein take on its characteristic interior and exterior or its overall globular character. Similar folding models have been proposed by other groups[7-9,14].

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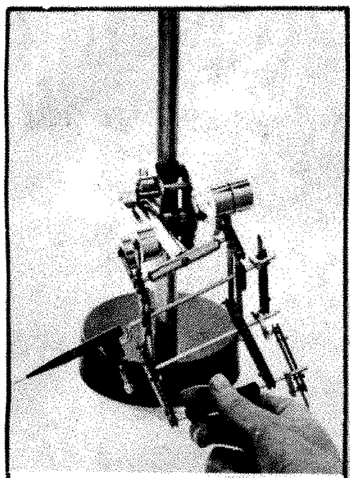
MINI-ADS



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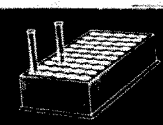

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reviews

Wealth of data on pulsars

S. Jocelyn Bell Burnell

Pulsars. By F. G. Smith. Pp. 239. (Cambridge University: Cambridge, London, New York and Melbourne, 1977.) £9.50.

THE tenth anniversary of the discovery of pulsars occurs later this year and at last the spate of papers seems to be slackening. It is a good time to produce a book on pulsars and we must be grateful to Professor Graham Smith for taking the trouble to do so. This book aims to be a summary and a review of the subject and is at a level suitable for research students in the field, or more senior scientists from other branches of astronomy who wish to have a reference book on the subject.

Its emphasis is mainly on the wealth of observational data that now exists on pulsars, with enough of the theoretical work included to give an idea of how well it all fits together. (It doesn't.) It describes phenomena rather than the theories which are current. There are some topics which the stranger to the subject might not expect to find in a book with this title—for example, several chapters on the interstellar medium, and some X-ray astronomy too. But they are rightly included. I have seen the contents page of another book on pulsars to be published later this year in the USA and it is very striking how similar both these books will be in the topics they choose to consider. Not only is it felt elsewhere too that the time is right to write this sort of book, but the scope of a book on pulsars is clearly determined by the state of the subject today.

The state of the subject is a mass, perhaps an embarrassment, of diverse observational results. The phenomena observed in pulsars are now well authenticated, although it is still hard to see whether differences are differences of degree or differences of kind. Our understanding as to *why* pulsars are as they are is much more restricted, and it could be some years hence before theoreticians solve some of the horrible problems presented by pulsars. Meanwhile, observers are anxious to find the critical experiments that should be made.

Although the pace has slackened, there have been some significant developments over the past few years which the author deals with in a variety of ways. For example, the recent conclusion that there is an embarrassingly large number of pulsars to have been formed in supernova explosions gets mentioned a few pages from the end; but throughout most of the book the impression is given that this is undoubtedly the way neutron stars are formed. (In fact one general criticism is that sometimes Professor Smith makes what may be tentative conclusions sound too established.)

The sections on X-ray observations of neutron stars have suffered most. The whole subject is changing very rapidly; even while writing the book Professor Smith admits he had trouble keeping up with it! A couple of years later these sections look very sorry. It must be difficult to judge how and where to draw the line when writing a book containing such material, but one option is not to attempt to include it in any detail. A similar restraint has been necessary until recently on the whole subject of pulsars.

One would like to recommend this book to a new research student but, although I warmly commend the radio astronomy (the greater part of the book), I would feel obliged to warn him that the X-ray and γ-ray sections are not to be relied upon as accurate accounts of the subjects. There are a number of misprints in the book as a whole which would drive a research student to exasperation, but sorting them out could be quite an educational process in itself. The index on occasion drove me to exasperation. (No Poincaré sphere, Professor Smith!)

The book is very readable with an informal style; it is a welcome compendium of the work that has been done by radio astronomers on and with pulsars. Many of us will find it a useful book for ourselves or others to refer to. We have had to wait a long time for it. ☐

S. Jocelyn Bell Burnell is a research fellow at the Mullard Space Science Laboratory, University College, London, UK.

Decade of the mouse

Development in Mammals. Vol. 1. Edited by Martin H. Johnson. Pp.386. (North-Holland: Amsterdam, New York and Oxford, 1977.) $34.50.

Now that the genetic code has been not only cracked but put to work, the last major uncharted territories of biology are usually reckoned to be the brain and development. The earlier years of the century saw a series of classical experiments on the embryology of invertabrates—for example, sea-urchins and lower vertebrates, in particular amphibians. The past ten years have been the 'Decade of the Mouse'. A wide sweep of technical advances—biochemical, biophysical, microsurgical, and above all advances in the techniques of *in vitro* culture—have rendered the mammalian embryo accessible for the first time to sophisticated analysis. The rapid strides that have been and are being made in our understanding of mammalian development, at the level of mechanisms as well as description, render the field immensely exciting to those of us working in it.

It is therefore timely that a new review series, *Development in Mammals*, has been launched. The editor is Martin Johnson, well known for his personal contribution to our understanding of the immunology of early mammalian embryos; the first two volumes "will concentrate on consideration of peri-implantation embryogenesis and the maternal influences on the embryo".

Volume 1 contains an interesting mix of developmental biology and reproductive physiology, two disciplines that in the past, in spite of their obvious overlap, have had inadequate contact. Reproductive physiologists have concentrated on the female and the male, often forgetting that reproduction implies an embryo too; developmental biologists, because of their traditional pre-occupation with non-mammalian material, sometimes find it hard to come to grips with the endocrinological, physiological and anatomical complexities of the maternal–embryonic relationship in mammals. This volume should go far to bridge the gap.

The biochemical and biophysical aspects of pre-implantation development are comprehensively covered by T. Ducibella (surface changes and cell–cell interactions in trophectoderm), R. M. Borland (transport processes in the mammalian blastocyst, the trophectoderm as an epithelium), G. A. Schultz and E. B. Tucker (protein synthesis and gene expression) and C. M. Warner (RNA synthesis and RNA polymerase activity). On the reproductive side, J. E. O'Grady and S. C. Bell provide an exhaustive review of uterine aspects of implantation; M. A. H. Surani describes protein changes in uterine fluids at the time of implantation; R. J. Aitken deals with delay of implantation, and the signals that may switch embryo and uterus on and off; M. Beato discusses the synthesis of one particular uterine protein, uteroglobin. M. H. Kaufman reviews his work on the effects of anaesthetics on gametogenesis and post-implantation development, in the context of clinical studies.

It is sad that the only hint of the intellectual excitement of the field, and of the beauty and amazing ingenuity of the mammalian embryo, comes in the Editor's introduction. The articles themselves tend to get bogged down in experimental detail, often contradictory. One consequence of selecting authors who are themselves actively working in a rapidly moving area is that one gets up-to-the-minute information, much of it still unpublished; a less desirable consequence is that the viewpoint is inevitably one-sided. Some of the articles are definitive reviews of a topic (Aitken; O'Grady and Bell) but most are of the nature of progress reports. Such a collection requires stringent editing. Able research workers are not necessarily good writers; Dr Johnson fields an impressive team but allows them too much freedom.

Many linguistic errors and misprints mar the pages: I spotted 54 in one 80-page article, without picking any editorial nits over hyphens or punctuation. The heterogeneity of misprint-density suggests that proof-reading was left to individual authors. There are also infelicities such as "Neither results strongly support . . . "; all 'less than' signs are omitted from probability levels in one chapter; and why choose a typeface that distinguishes l from 1 if you then abbreviate chlorine as Cl throughout? The typed text is photographically reduced to a size that I (and others to whom I showed the book) found tiring to read.

These are minor criticisms. I wholeheartedly welcome the appearance of the new series, and congratulate publishers and Editor on their enterprise. I urge anyone with an interest in developmental biology or reproductive physiology to buy this volume, and to get their libraries to buy it too. It is worth the money for the comprehensive reference lists as well as for the articles themselves.

Anne McLaren

Anne McLaren is Director of the MRC Mammalian Development Unit at University College, London, UK.

Antinucleon physics

Antinucleon–Nucleon Interactions. Edited by G. Eskpong and S. Nilsson. Pp. xvii+600. (Pergamon: Oxford and New York, 1977.) £22.

THE ANTIPROTON was discovered in 1955 and the antineutron a year later, so antinucleons have only recently come of age. The interaction of these particles with nucleons is an active research field, and in the past few years has been the subject of a number of specialised symposia of which the latest was held in Stockholm in July 1976. These published proceedings of this meeting contain 8 invited review papers and about 50 research contributions. The discussion following each talk has not been recorded.

The existence of antiparticles suggests the intriguing possibility of bulk antimatter in the Universe. A cosmology symmetric between matter and antimatter has been championed for a number of years by H. Alfvén (Stockholm) who reviewed this topic. There is no evidence for galaxies or other distant regions of antimatter, but neither can their existence be ruled out from direct experimental measurements.

All other talks came down to Earth and covered aspects of antinucleon behaviour in the laboratory. The construction of separated antiproton beams of high intensity, in particular at the CERN and Brookhaven proton synchrotons, has greatly enhanced the scope of low and medium energy experiments in the last few years. Antiprotons transported at a few hundred MeV/c can be brought to rest in targets and used to study the $\bar{N}N$ system at very low energies. This topic was reviewed by T.E.O. Ericson (CERN). Theoretical models predict the existence of $\bar{N}N$ states bound by the strong nuclear interaction, but these have not been established definitely by experiment. Antiprotons can, however, certainly be bound to nuclei by the Coloumb force to form antiprotonic atoms. X-ray transitions between levels have been studied and a number of interesting results obtained including a measurement of the antiproton magnetic moment.

Formation experiments are a powerful technique for studying hadron resonances. At the appropriate centre of mass energy, the colliding particles coalesce briefly to create a single particle, which then decays. To study posible meson formation, a conventional nucleon target requires a beam of antibaryons, in practice antiprotons. A clear review by E. Eisenhandler (Queen Mary College, London) outlined the considerable recent progress in this field. A few years ago suggestive enhancements were seen at various mass values in the antiproton–nucleon total cross sections. More recently these bumps were seen also in the $\bar{p}p$ elastic channel. The investigation of other channels, in particular two-meson final states provides much additional information. Angular distributions for $\bar{p}p\rightarrow\pi^0\pi^0$; $\eta^0\eta^0$; $\pi^0\eta^0$ were presented by a Bari–Brown–MIT collaboration, and results for polarisation in $\bar{p}p\rightarrow\pi^+\pi^-$; $K^+ K^-$ by a QMC–Daresbury–Rutherford collaboration.

When the Fermilab accelerator started operation, antiprotons in the 100 GeV/c region were produced quite copiously. At these momenta, however, particle separation using electrostatic or even RF fields becomes prohibitively difficult. An ingenious scheme has been used where antiprotons arising from antilambda decay in a short neutral beam were then transmitted to the experiment. At 100 GeV/c, annihilations are reduced to perhaps only a tenth of all $\bar{N}N$ interactions. These annihilations were reviewed by J. G. Rushbrooke (Cambridge). Experiments are still exploratory, measuring multiplicities, topological cross sections and inclusive production spectra. Annihilations at medium energies, and related processes, were covered by J. R. Fry (Liverpool). High energy non-annihilation interactions, where baryon and antibaryon are still present in the final state, were reviewed by S. Nilsson (Stockholm).

Two invited theoretical reviews were given. H. Miettinen (SLAC) discussed progress in antiproton physics mostly within the framework of quarks and duality diagrams. Both he, and also G. F. Chew (Saclay and Berkeley) in a more speculative review, considered the possibility of 'baryonium' states which do not have the conventional quark–antiquark structure of mesons.

Some plans for future facilities were outlined in contributed papers, but unfortunately the symposium came a little too early to include recent ideas on antiproton beam cooling and the possibility of storing antiprotons and colliding them with protons, thus giving rise to collisions at superhigh energies.

The editors and publisher have produced an attractive volume which should be of considerable interest to specialists in the field. **P. I. P. Kalmus**

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Platelet research

Platelets in Biology and Pathology. Edited by J. L. Gordon. Pp. 388. (North-Holland: Amsterdam and New York, 1977.) $53.95; Dfl. 132.

THIS book is the first volume in a new series *Research Monographs in Cell and Tissue Physiology,* with J. T. Dingle as general Editor. This volume, written by recognised cognoscenti in the various areas of platelet research, may be considered as a collection of progress reports over the past decade's research, with emphasis on current work. J. L. Gordon is responsible for the excellent selective editing.

Few scientists, if any, working with platelets, can keep abreast of the progress in this very active and rapidly developing field. B. Maupins annotated bibliography on blood platelets, for example, contains about 1,300 references to work published in 1972. The authors of the present monograph have very effectively filtered the plethora of material and offer the reader a comprehensive survey of the present state of knowledge; they provide roughly 1,600 selected and useful references.

The book is divided into two parts. Part one comprises an outline of fundamental platelet reactions, part two deals with platelet constituents, cellular control mechanisms and their biological significance. The first chapter in part one, "Blood platelets as multifunctional cells" (J. L. Gordon and A. J. Milner) is a well written appetiser, very fitting to the contents of the book. I am convinced that scientists to whom platelet research may seem a somewhat restricted subject, will acquire by reading these pages an informed interest in the numerous biological phenomena in which platelets are involved. The remainder of part one deals with platelet adhesion and aggregation (Baumgartner and Muggli) and the platelet release reaction (MacIntyre).

The book's second part brings together a great deal of valuable information on the platelet plasma membrane (Jamieson and Smith), lipids (Deykin), filaments and microtubules (Crawford), various platelet receptors (Mills and Macfarlane), biogenic amines (Drummond), platelets and thrombin (Majerus *et al.*), connective tissue and platelets (Jaffe), β-thromboglobulin (Moore and Pepper), platelets in immunological reactions (Brown), prostaglandins (Smith and Silver), platelet proteinases (Ehrlich and Gordon) and platelet growth-stimulating factor (Ross *et al.*).

In general, the chapters are well balanced; they bring out points of significance and offer a great deal more than a mere catalogue of original sources of information. It is to be hoped that some of the messages of the book will be widely appreciated—namely, that we still know next to nothing about the physiological importance of the many *in vitro* platelet phenomena so eagerly studied at present; that enough information has accumulated on interspecies differences in platelet chemistry and behaviour to make generalisations difficult or impossible; and that animal models for the study of the pathogenesis of human pathological conditions involving platelets are therefore very difficult to establish.

I found this monograph stimulating and I recommend it to anyone wishing to acquire an overall impression of the major developments and current lines of thought in the platelet field.

O. Behnke

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Parasitic protozoa

Parasitic Protozoa. Vol. 1: Taxonomy, Kinetoplastids and Flagellates of Fish. Edited by Julius P. Kreier. Pp. xv+441. (Academic: New York and London, 1977.) $35; £24.85.

THIS volume is the first of a series of four which are planned to present, in all, 40 chapters written individually by experts in each particular field and covering mainly the parasitic Protozoa but including also a number of organisms which are not, or only doubtfully, Protozoa, such as *Anaplasma, Eperythrozoon* and *Pneumocystis.* This is not so incongruous as might appear at first sight, as these organisms resemble the Protozoa in multiplying in their vertebrate host. The scope is, in fact, a welcome change from the more usual association of the Protozoa with only the helminths under the blanket term 'parasitology'.

The present volume contains 10 reviews, rather variable in length from about 25 to about 75 pages. The first two deal with taxonomy—respectively, with the position of the phylum Protozoa in the Animal Kingdom and with the internal classification of the phylum. The remainder deal exclusively, or predominantly with kinetoplastid flagellates and are rather variously defined. There are two defined purely taxonomically—the reviews of *Leishmania* and *Trypanosoma cruzi. Trypanosoma* is otherwise treated in six reviews defined by pathogenicity in relation to host and to geographical distribution—those species causing diseasesin man and other primates, or in cattle in subsaharan Africa or elsewhere, and those species generally regarded as 'non-pathogenic'. Finally, there is a treatment of the flagellate parasites of fish in which kinetoplastids figure largely but which also include dinoflagellates and polymastigids.

All the reviews are very readable, comprehensive and accurate treatments and (assuming their scope) are excellent summaries of the present states of knowledge for each field. Within the space provided for each review it is not possible to go into much discussion of matters which are still controversial, and so some statements will inevitably seem dogmatic on wider reading. Nevertheless, the reviews are all excellent lead-ins to their subjects and will be very useful for students and for research workers. The book is well produced, referenced and indexed. Its cost, nearly £25, seems high, even in these times, as it is only rather sparsely illustrated with a few line drawings and photographs. **W. H. R. Lumsden**

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Vertebrate evolution

Problems in Vertebrate Evolution. Edited by S. Mahala Andrews, R. S. Miles and A. D. Walker. Pp. xi+411. (Academic: London and New York, 1977.) £18.50; $36.10.

THIS volume of thirteen papers by vertebrate palaeontologists is a mark of their respect and regard for Professor Stanley Westoll on the occasion of his retirement. The subjects covered are, like his own interests, centred on fishes but extend also into the functional anatomy of tetrapods.

The commonest theme is the problem of comparative anatomy, in the sense of the search for the methods of establishing homology between structures in different groups, and also the application of these methods. Schaeffer develops the interesting and basic hypothesis that the dermal skeleton is always the result of an interaction between the epithelium and the underlying mesenchyme, and that its different manifestations (as enameloid, enamel, dentine or membrane bone) are merely the results of changes in the timing, duration or number of steps in the series of morphogenetic events. In another important paper, Patterson concludes that, contrary to current belief, there is no interchangeability between membrane bones and cartilage bones (though the two may fuse together), and that superficial "dermal" bones must be distinguished from membrane bones, the latter ossifying deep in the mesoderm. Ørvig gives a comparative survey of the superficial dermal structures, toothlike in appearance though not in position or function, that he calls odontodes. Gardiner and Bertram discuss the braincases of two new palaeoniscids and conclude that the ventral cranial fissure represents the gap between the ossifications in the trabecular–polar bar and the basal plate. Thomson discusses the ontogeny of cosmine, and suggests that the pore-canal system of Palaeozoic fishes had an electroreceptive function. Finally, Whiting returns to the problem of the identification of the cranial nerves of cephalaspid fishes, and provides considerable evidence that Stensiö's numbering of these should be revised.

The often bewildering interplay between ontogeny, functional anatomy and phylogeny is well shown by Panchen's work on the variety of vertebral structure in early tetrapods, and by Mahala Andrews' paper on the implications of some new features in the axial skeleton of *Latimeria*—since we still know virtually nothing of it as a living creature, its frequent mention as a "living fossil" in popular articles is rather ironic! On a related topic, Parrington suggests that intercentra were retained in the neck of cynodonts to increase the flexibility of this region. Finally, Walker provides some new ideas on the vexed question of the relationships between the functional morphology of the pelvic girdles of birds and dinosaurs (and, by implication, the relationships of their owners).

There are three papers primarily concerned with systematic work. Miles and Young describe new ptyctodontid placoderms from Australia, and provide arguments for a new classification of placoderms in which the arthrodires (*sensu stricto*) and antiarchs are closely related to one another. Jarvik finds a surprising degree of similarity between the detailed cranial anatomy of *Acanthodes* and that of living sharks, and suggests that these two groups are far more closely related than had been thought. Finally, Carroll explores the implications of his recent work on the earliest known (Permo–Triassic) lizards, discussing the basic adaptive pattern and early evolution of the group; he also suggests that sphenodontids are more closely related to the lizards than to the rhynchosaurs.

The volume has an attractive format and a good index. None of the papers in it are trivial, most are well-written (though occasionally blemished by imperfect proof-reading), and the volume deserves a place on the bookshelf of anyone working on the comparative anatomy and evolution of fishes.

Barry Cox

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Ultraviolet photoelectron spectroscopy

Principles of Ultraviolet Photoelectron Spectroscopy. By J. Wayne Rabalais. Pp. xv+454. (Wiley Interscience: New York and London, 1977.) $36; £22.50.

THIS is really a very good book which will be useful to photoelectron spectroscopists for many years to some—no mean achievement in such a rapidly changing research field. The author has not fallen into the trap of undue concentration on spectra of specific molecules or indeed of paying special attention to particular research areas within the ultraviolet photoelectron spectroscopy (PES) field. Rather, there has been a deliberate policy of concentrating on the fundamental principles of the various effects which are encountered in PES and for once the book's title is a true reflection of the content. In my opinion, the author was also correct in concentrating on the vacuum ultraviolet aspects of photoionisation electron spectroscopy, a subject which provides direct information on the eletcronic structure of molecular ions, rather than diluting the contents in an attempted comprehensive electron spectroscopy text.

The detailed content of the book is significantly different from that found in existing texts, and the degree of overlap is remarkably low. A suitably short introduction is followed by a brief but adequate chapter on experimental methods. Chapter three is a useful one in that it brings together, in a mathematical way, a number of related topics such as photoionisation, Franck–Condon factors, isotope effects, selection rules and configuration interaction. The next chapter deals with the theoretical methods used to describe and quantify ionisation from closed-shell molecules. This chapter is adequate, although a colleague thought that a discussion of the more recent molecular orbital methods such as the *ab initio* many body approach, the equations of motion method and the application of time-independent perturbation theory to the problem of calculating ionisation energies could have been included to advantage.

The next three chapters are ones which are not standard content in books on PES and for this reason are especially useful to researchers in the field. Chapter five deals with the special aspects of ionisation from open-shell molecules in a mathematical fashion, and includes a good account of the vector-coupling method for open shells. Chapter six deals with the theory of photoionisation cross-sections in as reasonable a way as is at present possible, since considerable development work is still required in this area. The following chapter deals with the interpretation of photoionisation cross-sections and angular distributions within the limits of our present day understanding.

Chapter eight is a detailed account of spin-orbit coupling in molecular ions and chapter nine is concerned with configurational instability of molecular ions—basically, the Jahn–Teller and Renner effects. On balance, the topics in chapter nine are discussed in rather more detail than one might justify from their importance in experimental PES. The last two chapters are more conventional and are basically concerned with experimental results and their interpretation.

Neville Jonathan

Neville Jonathan is Professor of Chemistry at the University of Southampton, UK.

Recent scientific and technical books

Applied Biology

BAKHLE, Y. S., and **VANE**, John R. (edited by). Metabolic Functions of the Lung. (Lung Biology in Health and Disease, Vol.4.) Pp.xvi + 353. ISBN-0-8247-6383-1. (New York and Basel: Marcel Dekker, Inc., 1977.) SFr.112.

BARLTROP, Donald (edited by). Paediatric Implications for Some Adult Disorders. (Scientific Proceedings of the 4th Unigate Workshop held at the Royal College of Physicians, St. Andrew's Place, NW1, May 1976.) Pp.vii + 152. ISBN-0-9501839-3-8. (London: Fellowship for Postgraduate Medicine, 1977.) £4.

BAUR, Rolf. Morphometry of the Placental Exchange Area. (Advances in Anatomy, Embryology and Cell Biology Vol.53, Part 1.) Pp.65. ISBN-3-540-08159-3. (Berlin and New York: Springer-Verlag, 1977.) DM32; $14.10.

BECK, William S. (edited by). Hematology, Second edition. Pp.xi + 589. ISBN-0-262-52038-9. (Cambridge, Mass. and London: The MIT Press, 1977.) $9.95.

BECKER, Frederick F. (edited by). Cancer: a Comprehensive Treatise. Vol. 5: Chemotherapy. Pp.xxi666. ISBN-0-306-35205-2. (New York and London: Plenum Press, 1977.) $51.

BERGSMA, Daniel, and **LENZ**, Widukind (edited by). Morphogenesis and Malformation of the Limb. (The Second International Conference on Morphogenesis and Malformation, held at Titisee, Federal Republic of Germany, Sponsored by The National Foundation—March of Fimes.) (Birth Defects: Original Article Series, Vol. 13, No.1.) Pp.xi + 364. ISBN-0-8451-1008-X. (New York: Alan R. Liss, Inc., 1977.) $35.

BIRCH, Gordon G., **SPENCER**, Michael and, **CAMERON**, Allan G. Food Science. Second edition. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.viii + 191. ISBN-0-08-021347-2. (Oxford and New York: Pergamon Press, 1977.) Hard cover £6.95; Flexi coves £3.95.

BLANDAU, Richard J., **BERGSMA**, Daniel (edited by). Morphogenesis and Malformation of the Genital System. (The Third International Conference on Morphogenesis and Malformation, held at Lake Wildnerness, Washington, July 1976.) Sponsored by The National Foundation—March of Dimes.) (Birth Defects: Original Article Series, Vol. 13, No.2.) Pp.xi.1 + 61. ISBN-0-8451-1009-8. (New York Alan R. Liss, Inc.

BLOEMENDAL, Hans (edited by). Cell Separation Methods. (Workshop on Methods in Cancer Research held in Lunteren, The Netherlands on November 11-12, 1976, organized under the auspices of the Scientific Council of The Netherlands Cancer Society (Koningin Wilhelmina Fonds).) Pp.v + 176. ISBN-0-7204-0649-8. (Amsterdam, New York and Oxford: North-Holland Publishing Company, 1977.) Dfl.73; $29.95.

BREWER, George J., and **PRASAD**, Ananda S. (edited by). Zinc Metabolism: Current Aspects in Health and Disease. (Proceedings of a Symposium sponsored by Meyer Laboratories Institute of Research, Fort Lauderdale, Florida, November 11-12, 1976.) (Progress in Clinical and Biological Research, Vol. 14.) Pp.xi + 365. ISBN-0-8451-0014-9. (New York: Alan R. Liss, Incl, 1977.) $26.

BROWN, Stanley S. (edited by). Clinical Chemistry and Chemical Toxicology of Metals. (Proceedings of the First International Symposium organized by the Commission on Toxicology, IUPAC Section on Clinical Chemistry, held at Monte Carlo, 2-5 March, 1977.) Pp.xi + 398. ISBN-0-444-41601-3. (Amsterdam, New York and Oxford: Elsevier/North-Holland, 1977.) Dfl.96; $39.25.

CARTER, Barbara L., **MOREHEAD**, **JAMES**, **WOLPERT**, Samuel M., **HAMMERSCHLAG**, Steven B., **GPIFFITHS**, Harry J., and **KAHN**, Paul C. Cross-Sectional Anatomy: Computed Tomography and Ultrasound Correlation. ISBN-0-8385-1255-0. (New York: Appleton-Century-Crofts, 1977. Distributed in UK by Prentice-Hall International, Hemel Hempstead.) £34; $54.

CHAN-PALAY, Victoria. Cerebellar Dentate Nucleus: Organization, Cytology and Transmitters. Pp.xxi + 548 (79 plates). ISBN-3-540-07958-0. (Berlin and New York: Springer-Verlag, 1977.) DM239; $105.20.

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CHRISPEELS, Maarten J., and **SADAVA**, David. Plants, Food, and People. (A Series of Books in Biology.) Pp.278. ISBN-0-7167-0378-5. (San Francisco: W. H. Freeman and Company, 1977.) Cloth $12; Paper $6.50.

DICKINSON, C. J. A Computer Model of Human Respiration: Ventilation—Blood Gas Transport and Exchange—Hydrogen Ion Regulation. Pp.xii + 256. ISBN-0-85200-173-8. (Lancaster: MTP Press, Ltd., 1977.) £14.95.

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MURPHY, Gerald P. (editor-in-chief). HLA and Malignancy. (Proceedings of a Symposium presented by Roswell Park Memorial Institute, Buffalo, New York, August 19-20, 1976.) (Progress in Clinical and Biological Research, Vol. 16.) Pp.xv + 248. (New York: Alan R. Liss, Inc., 1977.) np.

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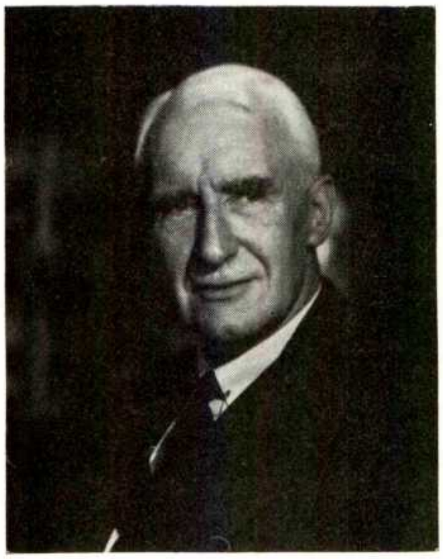
obituary

A. V. Hill

A. V. Hill died at his home in Cambridge on 3 June, in his 91st year. A.V., as he was known to his colleagues all over the world, was one of the great scientific leaders of his generation. He will be remembered not only for his pioneering work on the energy exchanges in active nerve and muscle, but for bringing physico-chemical ideas and precision measurements to bear on biological problems, and above all for his personal example and influence in inspiring and encouraging many of his younger colleagues, and in helping those who had been driven from their laboratories by political or racial persecution.

He was born at Bristol on 26 September 1886, went to Blundell's School at Tiverton, and in later years maintained a close connection with the West Country. He used to spend many summer months in Devonshire, staying with his family at 'Three Corners', his little house in Ivybridge, and working from time to time at the nearby Marine Biological Laboratory at Plymouth. In 1913, A.V. married Margaret Keynes, sister of John Maynard and Geoffrey Keynes, starting a partnership which lasted for nearly 60 years, and a pleasant family home whose hospitality is remembered by colleagues and friends from many countries.

At Blundell's School, he was profoundly influenced by J. Thornton, an outstanding mathematics teacher who also taught his pupils the need for clarity in thought and precision in the use of words. In 1905, A.V. went to Trinity College, Cambridge as a Scholar, taking mathematics and finishing as 3rd Wrangler in 1907. Under the tutelage of W. M. Fletcher (later the first Secretary of the Medical Research Council), he decided to turn to physiology and in 1909 took a first in part II of the Natural Sciences Tripos. The year after, he was made a Fellow of Trinity College.



In the Physiological Laboratory at Cambridge, A.V. found himself in the company of outstanding people, among them Joseph Barcroft, Keith Lucas, J. N. Langley, Walter Fletcher and F. Gowland Hopkins. He started by publishing theoretical papers on nerve excitation and on the kinetics of the oxygen-haemoglobin reaction. The latter is still referred to on occasions, when authors use the so-called 'Hill-coefficient' as a characteristic index in aggregate or cooperative molecular interactions. But before long, A.V. embarked on his own experimental research which he pursued, with interruptions caused by two world wars, for the next 55 years.

He followed Langley's advice to investigate 'the efficiency of the cut-out frog's muscle as a thermodynamic machine.' This became an unending story, in which he was helped by his assistants A. C. Downing and J. L. Parkinson, developing instruments of increasing sensitivity, greater time resolution and better stability, a story of continuously searching for errors in technique and interpretation, of replacing obsolete results and ideas with better experiments and fresh hypotheses; it was a process of temporary setbacks and continuously improved experimentation that gave A.V. the greatest pleasure and provided him with the kind of entertainment he enjoyed throughout his life. His early measurements of heat production in active muscle, analysing and dividing it into an initial anaerobic, and a subsequent, oxygen-requiring recovery phase, correlating them with what was known about lactic acid production and oxidative restoration processes, is now part of classical history, but at the time it was a most exciting technical and experimental advance and represented one of the outstanding contributions to our knowledge. Moreover, A.V. was quick in extending the work and applying it to the study of muscular exercise and the associated oxygen requirements in man.

Hill's preoccupation with precise and exceedingly sensitive myothermic measurements had some important 'spin-off.' It enabled him to detect the heat production during the conduction of nerve impulses, a feat which many distinguished scientists, among them the great Helmholtz, had attempted, but failed to bring off. In addition, A.V. showed that his thermopiles could be applied to practical problems, in measuring osmotic pressure of very small volumes of body fluids, and in determining arterial elasticity and pulse wave velocity. Although he once publicly admitted that he did his work 'not because it is useful, but because it's amusing', he was nevertheless accustomed to looking at living machinery with the eyes of a practical engineer. This, together with his personal interest in athletics, prompted him to study muscular movement not only in isolated frog muscles, but in man, and to work out the relations between physical dimensions of different animals and the limits to the speed and power of their muscular performance.

Hill's early work in the Cambridge laboratory came to a halt in 1914; he joined the Cambridgeshire Regiment, served as Captain and Brevet Major and later became Director of the Anti-Aircraft Experimental Section, another useful outlet for his engineering interests. He recommenced his muscle experiments in Cambridge at the end of the war, but in 1920 went to the Physiology Chair in Manchester. Three years later, he moved to University College London, first as Jodrell Professor of Physiology, then as Foulerton Professor of the Royal Society. He stayed at UCL, working in the laboratory for many years after his official retirement, until 1967 when he returned to Cambridge, leaving behind a Biophysics Department which he had founded in 1951.

A. V. Hill gave public service of many different kinds. During the uneasy years before 1939, he joined the Tizard Committee which initiated radar defence, and he also accepted the task of compiling a Central Register of Scientific and Technical Personnel in anticipation of wartime needs. He did this during a period when he was fully engaged in his laboratory, working single-handed on new experiments which resulted in the publication of one of his most important papers, on the energy liberation and the heat produced during shortening of active muscle. At the same time, he served as Biological Secretary of the Royal Society and on numerous scientific committees, and took a leading part in helping refugee scientists.

During the 1939/45 war he was Member of Parliament for Cambridge University, and he served as Scientific Adviser to the Government of India in 1943-44. To name only some of his other extramural activities, he served the Physiological Society, on its Committee, and as an Officer and Editor of its Journal, for 25 years, was Foreign Secretary of the Royal Society, Secretary General of the International Council of Scientific Unions, President of the British Association in 1952 when he gave his memorable address on 'The Ethical Dilemma of Science', and Chairman of the Society for the Protection of Science and Learning. A.V. gave a powerful stimulus to the study of marine biology in this country. He was President of the Marine Biological Association, and he constantly encouraged his younger colleagues to work at Plymouth and take advantage, not only of its delightful surroundings, but of the unique opportunities for experiments provided by the marine fauna. This initiative, together with his fascinating Liversidge Lecture on 'Chemical Wave Transmission in Nerve' helped to attract a number of distinguished biologists to the Plymouth Laboratory which later on led to very striking developments in the neurophysiological field.

A.V. was honoured by academic institutions all over the world; he was made a Companion of Honour in 1946 and received high honours from the Governments of France and the United States. At the age of 31 he was elected to the Royal Society who later awarded him the Royal and Copley Medals, and he received the 1922 Nobel Prize for Physiology together with Otto Meyerhof.

He is remembered and held in affection by all his pupils, not only as a great scientist who imparted to them the joy and spirit of adventure in doing experiments, but as a man who gave an example of uncompomisingly straight dealing, in the laboratory as well as outside. The pursuit of truth was to him not simply a scientific exercise, confined to one's work in the laboratory and presentation of results; it also governed his attitude in all his actions and in his relations to other persons. He was unrelentingly critical and never ceased to probe for flaws in his own deductions or experimental approach. 'To be uncritical', he said, 'particularly of oneself and one's ideas and motives, is the first long step towards dishonesty.' Yet, he never permitted his intellectual austerity to damp his enthusiasm, and I do not believe he ever allowed the slightest doubt to arise in his mind about the importance of the work he was engaged in. This buoyancy transmitted itself to those who were associated with him and helped to carry them over periods of failure and possible frustration. A.V. liked to get a good laugh out of situations which others might have regarded as perhaps slightly embarrassing; he detected and managed to focus on the humorous side of people's failings including his own. He retained, until nearly the end, a delightful, boyish sense of humour which greatly appealed to most of his friends, though at times it may have puzzled the more serious-minded ones among them.

I shall always remember a particular occasion in 1934. I was then a medical student in Leipzig, about to take my finals and anxiously making plans to escape from a hostile environment. I came across a correspondence in *Nature* between A. V. Hill and Johannes Stark, a famous physicist who had become the scientific 'Gauleiter' in Nazi Germany. After the 1914–18 war, A.V. had insisted (against some initial resistance) that colleagues from Germany should be invited straight away to attended International Congresses and be made to feel welcome in the scientific community. But in 1933 he was also the first to speak out vigorously against the Hitler regime and the dismissal and persecution of Jewish scientists. He was promptly taken to task by Professor Stark who stated in two letters to *Nature* that there was no factual basis for A.V.'s critical remarks, that the Nazi Government was obliged in self-protection to restrict the influence of certain persons and was simply engaged in lawful activities like any other respectable Government. The correspondence ended with a short note from A.V. in which he said that monetary contributions had been received in response to his appeal for assistance to colleagues who had been driven out of Germany. He added that he was not quite certain whether the donations were the result of his own eloquence, or rather of the explanations given by Professor Stark to whom, he felt some thanks were due. The *Nature* correspondence gave me my first impression, from a distance, of A.V.'s personality, and I found it so attractive that I made every effort to go and work with him as soon as I could.

There will be hundreds of scientists from different continents who recall with gratitude the welcome and hospitality they received, not only in the laboratory in Gower Street, but from A.V. and Margaret at their home in Highgate and at 'Three Corners' in Devonshire. Last year, on his 90th birthday, well over a hundred of his friends sent him a large volume containing greetings and reminiscences, and A.V. very much enjoyed browsing through them during the remaining months of his life. There can be few scientists and teachers who have won as much affection as A.V., and I believe that his personal influence among his scientific descendants in all parts of the world is a contribution to international scientific relations, not on a large scale, but of a kind perhaps more valuable than anything that official organisations could provide.

B. Katz

Roman Kozlowski

PROFESSOR ROMAN KOZLOWSKI, who died on 2 May, 1977, at the age of 88, was perhaps the most internationally honoured doyen of invertebrate palaeozoology. He was responsible for establishing a school of palaeontological study at Warsaw of exceptional breadth and excellence. His major contributions were on the Brachiopoda and Graptolithina, but he made some contributions on most other invertebrate fossil groups.

He was born on 2 February, 1889, at Wloclawek, north-west of Warsaw, Poland, and in 1907 he attended the University of Freiburg, and continued his geological and biological studies at the Sorbonne where he graduated in 1910. Between 1913 and 1921 Kozlowski was Professor of Geology and Mineralogy and later Director of the School of Mines at Oruro, Bolivia. It was during this period that his early work on the Carboniferous Brachiopoda and Devonian faunas of Bolivia was mostly done. The latter field he developed for a doctoral thesis under Professor Marcellin Boule (whom he affectionately spoke of as 'mon Maitre') which he received in 1923: the monograph, published in the *Annales de Paléontologie*, set a new standard for palaeontological illustration by collotype.

In 1924 he returned to Poland to teach at the University of Warsaw, becoming a full Professor of Geology and Palaeontology in 1934 and Chairman of the Palaeontology Department. Then really began the progressive growth to excellence of his department. His first major subsequent work followed visits in 1925 and 1926 to the Dneiper River sections in Podolia. The resulting monograph *Les brachiopodes gotlandien de la Podolie polonaise*, has been described by Shirley as 'a source of inspiration for a generation'; but this referred to the impeccable standard set by his brachiopod descriptions, especially of their internal structures. It was seminal in another way, since it gave details of a marine sequence passing from the Silurian into the Devonian. The area has recently been critical for the international definition of the Silurian/Devonian boundary.

In 1931 he noticed that graptolites were preserved in translucent chalcedony in Tremadocian rocks of the Holy Cross Mountains, and he discovered how easily these could be extracted by dissolution in hydrofluoric acid. This led eventually to the discovery of three new groups of graptoloid, and the discovery of much new detail on the ontogeny of the dendroid graptolites, and also the development of immaculate serial sectioning techniques. It also led to the extension of the techniques to other periods and a corresponding range of contributions on other groups.

The story of the survival of Kozlowski's manuscript for his monograph on Polish Tremadocian graptoloids is a most harrowing tale. His work was completed in 1939 and the bombing of Warsaw imperilled his laboratory, so he moved the typescript, illustrations and most of the specimens to a basement below the Seismological Conservatoire: his institute was destroyed. The Conservatoire was also destoyed, but a month later he was able to enter the basement, but found it sacked, and his material missing. Several months later he found part of his original manuscript in the university ruins, and shortly after, his friend Dr Rozkowski, who was later assassinated, discovered the remainder buried under snow in a courtyard.

At the Warsaw uprising of August 1944 Kozlowski was expelled, but first he hid his manuscript in the central heating system of a private house and, although the house was ruined, he was able to rescue the manuscript in 1945. The originals for his plates were lost, but since he had taken the precaution of sending negatives of these to Paris in 1939, he was able to publish the monograph in 1949. Immediately, this establshed a new reputation for him in the field of the graptoloids. Particularly important was the detailed case he argued for assigning this important fossil group to the Pterobranchia of the Hemichordata.

After the Second World War he returned to teaching and built up his research school again and until 1960, when he retired, he was Chairman of the Palaeontological Institute of the Polish Academy of Sciences in Warsaw. When the writer visited him in 1962 he was enthusiastically engaged in preparations for the Polish Mongolian expeditions which have led to so many valuable studies under the direction of his former student Professor Kielan-Jaworowska.

The Geological Society of London awarded him the Wollaston Medal in 1961; he was an honorary member of many Polish and international scientific societies, an honorary graduand of the universities of Krakow, Paris and Modena, a member of the Academies of Science of Poland, Czechoslovakia, France and Colombia; he was a Polish Scientific Prize-winner of the first degree.

The most important other monuments to the work of Roman Kozlowski are the two periodicals which he founded, namely, *Palaeontologica Polonica* of which he edited thirty-four volumes between 1929 and 1976, and *Acta Palaeontologica Polonica* which he edited from 1956 to 1974. These record the incredible breadth of research work done largely by himself and his students and research associates, and give an indication of his infectious enthusiasm for all branches of palaeobiology.

Michael House

George Cotzias

On June 13, 1977, just 3 days before his 59th birthday George Cotzias died of lung cancer. With his untimely passing the world lost a prominent neurobiological investigator who had made a number of fundamental contributions to the field.

Born in Athens, Greece, the son of a prominent political figure, he fled to the United States in 1941 when his country was occupied during World War II. His medical education which had begun at the University of Athens was continued at Harvard where in 1943 he was awarded the MD degree. Dr Cotzias then served as a house physician on the neurological and medical services of the Massachusetts General Hospital following which he began his career in research at the Rockefeller Institute in New York City. His initial interest centred on renal function but he soon became intrigued with the biological role of trace metals in tissue metabolism particularly in regard to the nervous system.

I remember well my first contact with Dr Cotzias in the 1950s. He was working on the mechanisms by which enzymes involved in oxidative phosphorylation were activated by manganese and how this might alter central nervous system function. I was organising a symposium on parkinsonism and invited him to discuss his work. Over a three-day period in the Ramapo Mountains of New York I came to know George's innovative and individualistic approach to science; his keen mind, his wit and gregarious yet unimposing nature.

He was then working at the Brookhaven National Laboratories to which he had moved in 1953 and where laboratory and clinical facilities were made available to him. Gathering a substantial team of investigators around him he began expanding his investigative program with particular interest in the biochemical mechanism underlying extrapyramidal and behavioural disorders. Stimulated by his previous work with manganese, as well as the growing body of information concerning the possible role of monoamines in melanin pigment formation, he was deeply involved in defining its role in Parkinson's disease. Though many were exploring the therapeutic value of monoamines particularly dopa in the treatment of parkinsonism, none approached it with the tenacity and courage of George Cotzias. Where others were constrained by adhering to the usual principles of therapeutics, he felt that the brain mechanisms were unique and required a more adventurous approach. The dramatic effects of reversing the symptoms of parkinsonism by giving large doses of L-dopa which were achieved by the Cotzias's group is undoubtedly one of the major milestones in the treatment of neurological disorders. Not only have literally millions with the disease benefited from its use, but the therapeutic principle has found applicability to other disorders. Despite being hailed by some as a panacea for Parkinsonism, Cotzias was the first to admit that it offered only a prolonged holiday from the symptoms and much more had to be done to effect a cure. Indeed, he devoted the last 10 years of his life to looking for more effective means of overcoming the dopamine deficiency in the brain of parkinsonians.

Dr Cotzias received many honours during his life, among which were the Albert Lasker Award in Clinical Medical Research in 1969, the Borden Award in 1972 and the Oscar B. Hunter Award from the American Society of Clinical Pharmacology and Therapeutics in 1973. His native country recognised his contributions with an Honorary Doctor of Medicine degree from the National and Kapodistrian University in Athens, and in appointing him Grand Commander of the Royal Greek Order of the Phoenix and the Archon Actuarius of the Ecumenical Orthodox Patriarchate.

At the time of his death he was Professor of Neurology at the Cornell Medical College, Attending Physician at New York Hospital and a Special Assistant to the President of the Memorial Sloan-Kettering Cancer Center. He had accepted these positions in 1974, moving his laboratories and personnel just prior to becoming ill. Despite this, he continued to be active and involved in a number of new fields of investigation related to the aging process in the nervous system and mechanisms involved in carcinogenesis. Undoubtedly, this work will be carried forward by his colleagues and collaborators, many trained by him over the years. Yet his presence will be sorely missed in the scientific community throughout the world.

Melvin D. Yahr

C. F. Hickling

Dr C. F. Hickling, CMG, ScD, who died on 14 June at the age of 74 had a unique influence on the development of marine, lake and river fisheries and fish culture over the wide extent of the former British Empire. He was educated at Taunton School and then at St. Catherine's College, Cambridge, to join, in 1927, the staff of the Fisheries Laboratory at Lowestoft. He was a born fisheries naturalist, as keenly interested in the methods of fishing and in the fishermen as in collecting data and interpreting their significance. His prime concern was with the hake fishery, to rise and then fall during this period. It has rightly been said that our knowledge of the biology of that fish owes everything to him. This formed the subject matter of a series of reports largely summarised in his Buckland lectures for 1934 on *The Hake and the Hake Fishery*.

During the war he was Port Fishery Officer at Milford Haven. In 1945 he was appointed Fisheries Adviser to the Secretary of State for the Colonies. In 1961, a year before his retirement, he was transfered to the Department of Technical Co-operation. His activities were literally world-wide, on innumerable islands in the Caribbean and Pacific, in East and West Africa, in Malaya, Singapore, Hong Kong and Sarawak. He advised on river fisheries in Nigeria, on those of the great lakes of Central Africa—then on those of the created Lake Kariba—and promoted development of sea fisheries off African and Pacific coasts.

He was responsible for the organisation of training courses for fisheries officers appointed during this belated period of colonial activity, also for advice on the location and planning of research laboratories notably at Entebbe in Uganda, in Zanzibar, on Lake Nyasa, at Freetown, Sierre Leone and at Singapore. The largest scheme, carried through during the communist troubles, was the Tropical Fish Culture Institute at Malacca of which, there happily accompanied by his wife, Marjory, he was Acting Director in 1957-59. There he succeeded in crossing strains of *Tilapia mossambica* to produce unisex cultures which grew without the runting that accompanies the early reproductive activities in these fish.

It was a notable experience, as the writer can testify, to accompany Fred on a tour. He moved from the Governor's residence to the humblest fish landing, advising on scientific and commercial policy and then on the fitting of outboard motors to dug-out canoes, on the quality and handling of nets and on preserving and marketing of the catch. He was rightly described as a 'one man FAO'.

He leaves much of his wide knowledge to posterity in the pages of his books on *Tropical Inland Fisheries* and *Fish Culture*. All who knew him remember him with deep affection, so utterly the right man in the right place. We think also of his widow and two sons.

Maurice Yonge

J. A. Robbie

James Andrew Robbie, FRSE, FGS, died in Edinburgh in his 67th year on 19 May 1977. Born near Laurencekirk he graduated BSc with Honours in Geology at Aberdeen University in 1934. After a short period as Demonstrator in the Geology Department there he was appointed Geologist in the Geological Survey of Great Britain in 1935. He served initially in southern England carrying out geological surveys in the vicinity of Chatham, Bridport and Yeovil. Publication of his work was delayed by the Second World War but eventually appeared as a paper on the Chalk Rock at Winterborne Abbas in his favourite county of Dorset and contributions to the Geological Survey Memoirs on Chatham and Bridport and Yeovil. Although concerned with water supply, mineral resources and airfield construction during the war he was located in London, a period of his life which evoked vivid tales of the blitz and food shortages, the latter being the greater burden for the gourmet.

In 1947 he was transferred to Belfast as part of the team which initiated the Geological Survey of Northern Ireland. On promotion to District Geologist in charge of the Belfast Office in 1959 he was instrumental in establishing the close relationships with central and local government which have resulted in a key role for the Geological Survey in the area. The acme of his geological career came in Northern Ireland with many studies including those of the Mourne Mountain granite as revealed in the Slieve Binnian Tunnel and of the Carboniferous stratigraphy proved by boreholes between Coalisland and Dungannon, published in the Bulletin of the Geological Survey of Great Britain. The scope of his experience and knowledge is demonstrated by his accounts of lithology, stratigraphy and structure of strata of the Dalradian, Silurian, Carboniferous, Triassic, Rhaetic, Liassic, Cretaceous, Tertiary and Pleistocene in memoirs describing the geology of the Dungannon, Ballycastle and Belfast districts.

When promoted to Edinburgh to be Assistant Director in charge of all the activities of the Institute of Geological Sciences in Scotland he was so deeply entrenched in Northern Ireland geology that his model became something of an anathema to the Scots in their analyses of local geology. While involved in the expansion of IGS activities both on land and offshore he played a major role in planning and developing Murchison House, the new headquarters of IGS in Scotland, which was nearing completion as he retired in 1975.

Elected a Fellow of the Royal Society of Edinburgh in 1968 he was appointed Vice President in 1975. He was a Fellow of the Geological Society of London, served as President of the Edinburgh Geological Society from 1971–73, and was elected an Honorary Member of the Belfast Geologists' Society of which he was President twice. In his official capacity he served on the Council of Management of the Macaulay Institute for Soil Research from 1969 until 1975.

A quiet, shy, self-effacing man Jim Robbie shunned the social scene and yet enjoyed nothing more than to meet friends over a glass of his favourite national spirit. He faced his retirement full of plans for remodelling his garden, refurbishing his beloved old car and travelling the world, plans only in the earliest stages of fulfillment when he died.

G. I. Lumsden

nature

Author Index for Volume 268

July–August 1977
Numbers 5615–5622
Pages 1–780

Adelstein R. S. *see under* Scordilis S. P., **268,** 558
Aguayo A. J., Kasarjian J., Skamene E., Kongshavn P. and Bray G. M. Myelination of mouse axons by Schwann cells transplanted from normal and abnormal human nerves, **268,** 753
Al-Ani S. *see under* Thompson E. J., **268,** 241
Ambraseys N. N. Long-period effects in the Romanian earthquake of March 1977, **268,** 324
Ananthanarayanan V. S. and Hew C. L. A synthetic polypeptide with antifreeze activity, **268,** 560
Anderson C. R., Cull-Candy S. G. and Miledi R. Potential-dependent transition temperature of ionic channels induced by glutamate in locust muscle, **268,** 663
Anderson J. R. and Howe R. F. Generation of a supported iridium catalyst of extremely high dispersion, **268,** 129
Andrews P. and Tobien H. New Miocene locality in Turkey with evidence on the origin of *Ramapithecus* and *Sivapithecus*, **268,** 699
Arnason A., Larsen B., Marshall W. H., Edwards J. H., Mackintosh P., Olaisen B. and Teisberg P. Very close linkage between HLA-B and Bf inferred from allelic association, **268,** 527
Arnold F. and Krankowsky D. Water vapour concentrations at the mesopause, **268,** 218
Atlas D., Hanski E. and Levitzki A. Eighty thousand beta-adrenoreceptors in a single cell, **268,** 144
Auffret G. A. *see under* Montadert L., **268,** 305
Augustus J., Bijman J., van Os C. H. and Slegers J. F. G. High conductance in an epithelial membrane not due to extracellular shunting, **268,** 657
Bada J. L. *see under* Masters P. M., **268,** 71
Badham J. P. N. and Stanworth C. W. Evaporites from the lower Proterozoic of the East Arm, Great Slave Lake, **268,** 516
Bailey J., Borer K., Combley F., Drumm H., Krienen F., Lange F., Picasso E., von Ruden W., Farley F. J. M., Field J. H., Flegel W. and Hattersley P. M. Measurements of relativistic time dilation for positive and negative muons in a circular orbit, **268,** 301
Baker C. R. B. *see under* Hull R., **268,** 402
Baker D. J. Mr, Nowlin W. D. Jr, Pillsbury R. D. and Bryden H. L. Antarctic circumpolar current: space and time fluctuations in the Drake Passage, **268,** 696
Baldissera F. and Campadelli P. How motoneurones control development of muscle tension, **268,** 146
Baltimore D. *see under* Pettersson R. F., **268,** 270
Banerjee S. P., Kung L. S., Riggi S. J. and Chanda S. K. Development of beta-adrenergic receptor subsensitivity by antidepressants, **268,** 455
Banfalvi Z. *see under* Kondorosi A., **268,** 525
Barbieri C., Romano G., di Serego S. and Zambon M. The optical variability of 3C345, **268,** 318
Barker C. R. *see under* Burns G. F., **268,** 243
Barnes R. A. Sulphur deposit account, *news,* **268,** 92
Barry D. C. Relation between metallicity and multiplicity for solar type stars, **268,** 509
Barry R. D. *see under* Spooner L. L. R., **268,** 650
Bartell L. S. and Johnson R. D. Molecular images by electron-wave holography, **268,** 707
Bartholomew J. C. *see under* Teng M-H., **268,** 739
Bartis F. J. Pre-melting near crystal dislocations, **268,** 427
Barton S. B. and Cohen I. S. Are transmitter release statistics meaningful? **268,** 267
Bash F. N. *see under* Wheeler J. C., **268,** 706
Bates D. L., Danson M. J., Hale G., Hooper E. A. and Perham R. N. Self-assembly and catalytic activity of the pyruvate dehydrogenase multienzyme complex of *Escherichia coli*, **268,** 313
Bauer W. *see under* Roemer D., **268,** 547
Beaugé, L. A. and Glynn I. M. A mofifier of (Na +K) ATPase in commercial ATP, **268,** 355
Beers G. V. *see under* Thompson E. J., **268,** 241
Bell G. U-shaped gene frequency distributions, *matters arising,* **268,** 374
Benemann J. R., Weissman J. C., Koopman B. L. and Oswald W. J. Energy production by microbial photosynthesis, *review article,* **268,** 19
Ben-Hur E. *see under* Heimer Y. M., **268,** 170
Benjamin P. R. *see under* Winlow W., **268,** 263
Bettany B. W. *see under* Murlis J., **268,** 433
Bevan E. A. *see under* Herring A. J., **268,** 464
Bibb M. J. *see under* Hopwood D. A., **268,** 171
Bijman J. *see under* Augustus J., **268,** 657
Birdwell C. R., Gospodarowicz D. and Nicolson G. L. Factors from 3T3 cells stimulate proliferation of cultured vascular endothelial cells, **268,** 528
Bischoff A. *see under* Meier C., **268,** 177
Bishop J. O. Lampbrush chromosomes brought up to date, *news and views,* **268,** 588
Bissell M. J. *see under* Teng M-H., **268,** 739
Bjorseth A. *see under* Lunde G., **268,** 518
Blake C. C. F. Towards some enzyme mechanisms, *news and views,* **268,** 485
Blake C. C. F. and Oatley S. J. Protein–DNA and protein–hormone interactions in prealbumin: a model of the thyroid hormone nuclear receptor? **268,** 115
Blythe C. and Rush H. What kind of food policy? *news,* **268,** 386
Bobek B. Summer food as the factor limiting roe deer population size, **268,** 47
Bock W. *see under* Montadert L., **268,** 305
ollenbacher W. E., Smith S. L., Wielgus J. J. and Gilbert L. I. Evidence for an alpha-ecdysone cytochrome P-450 mixed function oxidase in insect fat body mitochondria, **268,** 660
ll G. P., Callow J. A., Callow M. E. and Evans L. V. Cross-fertilisation in fucoid seaweeds, **268,** 626
Borer K. *see under* Bailey J., **268,** 301
Borys R. D. *see under* Rahn K. A., **268,** 713
Botley C. M. *see under* Edwards O., **268,** 565
Bradford P. M. *see under* Case B., **268,** 223
Braizer L. *see under* Skirball L. R., **268,** 352
Brandt K. *see under* Mattsson H., **268,** 52
Branton D. *see under* Fowler V., **268,** 23
Brasier M. D. An early Cambrian chert biota and its implications, **268,** 719
Bray G. M. *see under* Aguayo A. J., **268,** 753
Brennan B. J. and Stacey F. D. Frequency dependence of elasticity of rock — test of seismic velocity dispersion, **268,** 220
Brewers J. M. and Yeats P. AL Oceanic residence times of trace metals, **268,** 595
Brice K. A., Eggleton A. E. J. an Penkett S. A. An important ground surface sink for atmospheric nitrous oxide, **268,** 127
Briggs P. L. and Press F. Pattern recognition applied to uranium prospecting, **268,** 125
Brittenham G. Genetic model for observed distributions of proportions of haemoglobin in sickle-cell trait, **268,** 635
Britton G., Lockley W. J. S., Powls R., Goodwin T. W. and Heyes L. M. Carotenoid transformations during chloroplast development in *Scenedesmus obliquus* PG1 demonstrated by deuterium labelling, **268,** 81
Brodie E. D. Hedgehogs use toad venom in their own defence, **268,** 627
Bronchart R. and Demoulin V. Unusual mitosis in the red alga *Porphyridium purpureum*, **268,** 80
Brostoff S. W., Levit S. and Powers J. M. Induction of experimental allergic neuritis with a peptide from myelin P_2 basic protein, **268,** 752
Brown A., Nevo E. and Zohary D. Association of alleles at esterase loci in wild barley *Hordeum spontaneum* L., **268,** 430
Brown F. *see under* Sangar D. V., **268,** 648
Brown J. *see under* Lubs H. A., **268,** 631
Brown J. C. and Hughes D. W. Tunguska's comet and non-thermal ^{14}C production in the atmosphere, **268,** 512
Brown R. E. *see under* Hovanessian A. G., **268,** 537
see under Kerr I. M., **268,** 540
Bruckenstein S. Physicochemical hydrodynamics, *news and views,* **268,** 298
Bryden H. L. *see under* Baker D. J. Jr, **268,** 696
Brzezinski W. *see under* Kaminiski M., **268,** 238
Buescher H. H. *see under* Roemer D., **268,** 547
Bunn Moreno M. M. *see under* Sanderson C. J., **268,** 340
Burford R. O. Bimodal distribution of creep event amplitudes on the San Andreas fault, California, **268,** 424
Burness A. T. H. *see under* Enegren B. J., **268,** 536
Burns G. F., Nash A. A., Worman C. P., Barker C. R., Hayhoe F. G. J. and Cawley J. C. A human leukaemic cell expressing hybrid membrane phenotypes, **268,** 243
Byus C. V., Klimpel G. R., Lucas D. O. and Russell D. H. Type I and type II cyclic AMP-dependent protein kinase as opposite effectors of lymphocyte mitogenesis, **268,** 63
Cadogan P. H. Palaeoatmospheric argon in Rhynie chert, **268,** 38
Cafruny W. *see under* Senitzer D., **268,** 158
Cain W. S. Bilateral interaction in olfaction, **268,** 50
Callow J. A. *see under* Bolwell G. P., **268,** 626
Callow M. E. *see under* Bolwell G. P., **268,** 626
Calvert P. NMR of macromolecules, **268,** 105
Calvert P. D. Stresses at spherulite boundaries, **268,** 321
Cameron D. J. and Erlanger B. F. Evidence for multispecificity of antibody molecules, **268,** 763
Campadelli P. *see under* Baldissera F., **268,** 146
Campbell S. *see under* Edwards O., **268,** 565
Cardinaux F. *see under* Roemer D., **268,** 547
Carney D. H. and Cunningham D. D. Initiation of chick cell division by trypsin action at the cell surface, **268,** 602
Carpenter R. G. and Emery J. L. Final results of study of infants at risk of sudden death, **268,** 724
Casanova J. *see under* Samuels H. H., **268,** 254
Case B., Bradford P. M. and Garbett K. Silicate and the aqueous corrosion of a high-magnesium alloy, **268,** 223
Cawley J. C. *see under* Burns G. F., **268,** 243
Chanda S. K. *see under* Banerjee S. P., **268,** 455
Chandler D. E. and Williams J. A. Fluorescent probe detects redistribution of cell calcium during stimulus–secretion coupling, **268,** 659
Chang S. Y. *see under* Hughes M. R., **268,** 347
Chapman C. S. *see under* Tobe S. S., **268,** 728
Chapman H. J. and Moorbath S. Lead isotope measurements from the oldest recognised Lewisian gneisses of north-west Scotland, **268,** 41
Chou J. Y., Robinson J. C. and Wang S-S. Effects of sodium butyrate on synthesis on human chorionic gonadotrophin in trophoblastic and non-trophoblastic tumours, **268,** 543
Christensen I. P. and Gregory A. H. Further study of an auditory illusion, **268,** 630
Chutna J. *see under* Hasek M., **268,** 68
Cifelli R. Atlantic palaeotemperatures during the Cainozoic — Reply, *matters arising,* **268,** 374
Clark G. W. *see under* Rappaport S., **268,** 705
Clarke C. A. *see under* Marsh N. A., **268,** 726
Clarke P. G. H. *see under* Ramachandran V. S., **268,** 333
Closse A. *see under* Roemer D., **268,** 547
Coe M. J., Engel A. R., Quenby J. J. and Dyer C. S. A line feature at 64 keV in the X-ray spectrum of Her X-1, **268,** 508
Coffino P. *see under* Gehring U., **268,** 167
Cogne J. *see under* Peucat J. J., **268,** 131
Cohen I. S. *see under* Barton S. B., **268,** 267
Cohen L. B. *see under* Grinvald A., **268,** 140
Cohen M. H., Kellermann K. I., Shaffer D. B., Linfield R. P., Moffet A. T., Romney J. D., Seielstad G. A., Pauliny-Toth I. I. K., Preuss E., Witzel A., Schilizzi R. T. and Geldzahler B. J. Radio sources with superluminal velocities, *review article,* **268,** 405
Cohen S. N. *see under* Hopwood D. A., **268,** 171
Collis P. S. *see under* Weeks D. P., **268,** 667
Combley F. *see under* Bailey J., **268,** 301
Comfurius P. *see under* Zwaal R. F. A., **268,** 358
Cook P. J. Loss of boron from shells during weathering and possible implications for the determination of palaeosalinity, **268,** 426
Cope J. C. W. An Ediacara-type fauna from South Wales, **268,** 624
Corley R. B. *see under* Kindred B., **268,** 531
Corsaro C. M. and Migeon B. R. Contact-mediated communication of ouabain resistance in mammalian cells in culture, **268,** 737
Costa E. *see under* Fratta W., **268,** 452
Cowie R. J. Optimal foraging in great tits (*Parus major*), **268,** 137
Coy D. H. *see under* Cusan L., **268,** 544
Coyne G. V. *see under* Hubbard W. B., **268,** 33
Crystal R. G. *see under* Hance A. J., **268,** 152
Cull-Candy S. G. *see under* Anderson C. R., **268,** 663
Cunningham D. D. *see under* Carney D. H., **268,** 602
Curtis D. R. *see under* Krogsgaard-Larsen P., **268,** 53
Cusan L., Dupont A., Kledzik G. S., Labrie F., Coy D. H. and Schally A. V. Potent prolactin and growth hormone releasing activity of more analogues of Met-enkephalin, **268,** 544
Dams L. *see under* Dams M., **268,** 228
Dams M. and Dams L. Spanish rock art depicting honey gathering during the Mesolithic, **268,** 228
Danson M. J. *see under* Bates D. L., **268,** 313
Darling J. L. *see under* Gaborak M., **268,** 339
Darmon M. *see under* Klein C., **268,** 76
Das Y. T. and Gupta A. P. Nature and precursors of juvenile hormone-induced excessive cuticular melanisation in German cockroach, **268,** 139
Davies J. and Dray A. Substance P and opiate receptors, **268,** 351
Davies P. C. W. Experimenting with controlled gravity, *news and views,* **268,** 397
Davis H. T. *see under* Larson R. G., **268,** 409
Dawson P. *see under* Wild A. E., **268,** 443
de Caro G. *see under* Evered M. D., **268,** 332
de Deckere E. A. M., Nugteren D. H. and Hoor F. T. Prostacyclin is the major prostaglandin released from the isolated perfused rabbit and rat heart, **268,** 160
De Weer P. *see under* Kennedy B. G., **268,** 165
Delibrias G. *see under* Talbot M. R., **268,** 722
Del Fiacco M. *see under* Di Chiara G., **268,** 743
Demoulin V. *see under* Bronchart R., **268,** 80
Dennis M. J. *see under* Sargent P. B., **268,** 456
Dermott E. *see under* Thorne H. V., **268,** 345
Deshpande M. R., Vats H. O., Sethia G. and Murthy B. S. Daytime inosphere scintillation associated with geomagnetic storms, **268,** 614
DeSombre E. R. *see under* Lyttle C. R., **268,** 337
Detjen B. *see under* Nomoto A., **268,** 208
Devlin J. C., Dyson P. L. and Hammer P. R. Small-amplitude, short-period waves in the E and F regions of the ionosphere, **268,** 319
Dhondt A. A. Interspecific competition between great and blue tit, **268,** 521
Di Chiara G., Olianas M., Del Fiacco M., Spano P. F. and Tagliamonte A. Intranigral kainic acid is evidence that nigral non-dopaminergic neurones control posture, **268,** 743
di Serego S. *see under* Barbieri C., **268,** 318
Dichter M. A., Tischler A. S. and Greene L. A. Nerve growth factor-induced increase in electrical excitability and acetylcholine sensitivity of a rat pheochromocytoma cell line, **268,** 501
Dingle R. V. and Scutton R. A. Continental margin fault pattern mapped south-west of Ireland, **268,** 720
Ditta G., Soderberg K. and Scheffler I. E. Chinese hamster cell mutant with defective mitochondrial protein synthesis, **268,** 64
Dorsch S. E. *see under* Hall B. M., **268,** 532
Douzou P., Debey P. and Franks F. Enzymology in supercooled water, **268,** 466
Dower R. *see under* Rappaport S., **268,** 705
Doxsey R. *see under* Rappaport S., **268,** 705
Dray A. *see under* Davies J., **268,** 351
Dretchen K. L. *see under* Skirboll L. R., **268,** 352
Drumm H. *see under* Bailey J., **268,** 301
Dubois E. L. *see under* Gardner M. B., **268,** 341
Dudley R. K. *see under* Lamb C. J., **268,** 692
Dunne J. J. *see under* Milner A. D., **268,** 175
DuPeuble P. A. *see under* Montadert L., **268,** 305
Dupont A. *see under* Cusan L., **268,** 544
Dyer C. S. *see under* Coe M. J., **268,** 508
Dyson P. L. *see under* Devlin J. C., **268,** 319
Earnshaw W. C. and Harrison S. C. DNA arrangement in isometric phage heads, **268,** 598
Eckert R. Genes, channels and membrane currents in *Paramecium*, **268,** 104
Edwards J. H. *see under* Arnason A., **268,** 527
Edwards O., Campbell S., Botley C. M., Flores N. R., Palmer T. J. and Hulse E. V. The Star of Bethlehem, *matters arising,* **268,** 565
Eggleton A. E. J. *see under* Brice K. A., **268,** 127

Ekins R. P. *see under* Marshall N. J., **268,** 58
Elder M. G. *see under* Myatt L., **268,** 159
Ellis R. A., Goertemiller C. C. Jr and Stetson D. L. Significance of extensive 'leaky' cell junctions in the avian salt gland, **268,** 555
Ellis R. H. *see under* Roberts E. H., **268,** 431
Emery J. L. *see under* Carpenter R. G., **268,** 724
Emley E. F. Magnesium and energy, *news and views,* **268,** 299
Endo T., Kuwahara A., Tasai H., Murata T., Hashimoto M. and Ishigami T. Intermolecular association and geometrical shape as two factors controlling chemical reactions, **268,** 74
Enegren B. J. and Burness A. T. H. Chemical structure of attachment sites for viruses on human erythrocytes, **268,** 536
Engel A. R. *see under* Coe M. J., **268,** 508
Eriksson A. J. Video tape recording of a possible ball lightning event, **268,** 35
Erlanger B. F. *see under* Cameron D. J., **268,** 763
Estridge M. Polypeptides similar to the alpha and beta subunits of tubulin are exposed on the neuronal surface, **268,** 60
Evans L. V. *see under* Bolwell G. P., **268,** 626
Evered M. D., Fitzsimmons J. T. and de Caro G. Drinking behaviour induced by intoacranial injections of eledoisin and substance P in pigeon, **268,** 332
Everitt B. J. *see under* Tye N. C., **268,** 741
Evgen'ev M., Levine A. and Gubenko I. Are late replicating regions in polytene chromosomes of *Drosophila* enriched by repeated nucleotide sequences? **268,** 766
Fabian A. C. X-ray bursts from dense clouds, **268,** 607
Fairbridge R. W. and Hillaire-Marcel C. An 8-000-yr palaeoclimatic record of the 'Double-Hale' 45-yr solar cycle, **268,** 413
Farley F. J. M. *see under* Bailey J., **268,** 301
Ferenczy L. and Maráz A. Transfer of mitochondria by protoplast fusion in *Saccharomyces cerevisiae,* **268,** 524
Fesenko E. E. and Lyubarskiy A. L. Effect of light on artificial lipid membranes modified by photoreceptor membrane fragments, **268,** 562
Field J. H. *see under* Bailey J., **268,** 301
Finch A. P. *see under* Taussig M. J., **268,** 178
Finch R. A. *see under* Paulsen D. F., **268,** 639
Fisher P. R. *see under* Pemberton J. M., **268,** 732
Flanegan J. B. *see under* Pettersson R. F., **268,** 270
Flegel W. *see under* Bailey J., **268,** 301
Flores N. R. *see under* Edwards O., **268,** 565
Florin-Christensen A. *see under* Marshall N. J., **268,** 58
Flowers K. Test of theballistic hypothesis of movement and the 'point of no return', **268,** 174
Flynn G. J. and Loubet M. Superheavy element fission hypothesis in carbonaceous chondrites, **268,** 717
Ford N. C. *see under* Potts R., **268,** 563
Forrai T. *see under* Kondorosi A., **268,** 525
Forsum U. *see under* Klareskog L., **268,** 248
Foster R. B. *Tachigalia versicolor* is a suicidal neotropical tree, **268,** 624
Fournier M. J. *see under* Potts R., **268,** 563
Fowler V. and Branton D. Lateral mobility of human erythro cyte integral membrane proteins, **268,** 23
Fox M. Environmental mutagenesis, *news and views,* **268,** 488
Frangione B. *see under* Levo Y., **268,** 56
Franklin E. C. *see under* Levo Y., **268,** 56
Fraser P. J. Cercal ablation modifies tethered flight behaviour of cockroach, **268,** 523
Fratta W., Yang H.-Y. T., Hong J. and Costa E. Stability of Met-enkephalin content in brain structures of morphine-dependent or foot shock-stressed rats, **268,** 452
Freimer E. H. *see under* Senitzer D., **268,** 158
Fujihira M., Ohishi N. and Osa T. Photocell using covalently-bound dyes on semiconductor surfaces, **268,** 226
Fujisaka M. *see under* Hotta S., **268,** 733
Gaborak M., Darling J. L. and Gutteridge W. E. Comparative drug sensitivities of culture forms of *Trypanosoma cruzi* and *Trypanosoma dionisii,* **268,** 339
Gallardo V. A. Large benthic microbial communities in sulphide biota under Peru−Chile Subsurface Countercurrent, **268,** 331
Galton P. M. The ornithopod dinosaur *Dryosaurus* and a Laurasia-Gondwanaland connection in the Upper Jurassic, **268,** 230
Garbett K. *see under* Case B., **268,** 223
Gardner I. D. *see under* Wing e. j., **268,** 642
Gardner M. B., Ihle J. N., Pillarisetty R. J., Talal N., Dubois E. L. and Levy J. A. Type C virus expression and host response in diet-cured NZB/W mice, **268,** 341
Gaskell W., Illingworth A. J., Latham J. and Moore C. B. Airborne studies of thunderstorm electrification, **268,** 124
Gates M. A. Analysis of positional information applied to cirral patterns of the ciliate *Euplotes,* **268,** 364
Gealt M. A. *see under* Weeks D. P., **268,** 667
Gehrels T. *see under* Hubbard W. B., **268,** 33
Gehring U. and Coffino P. Independent mechanisms of cyclic AMP and glucocorticoid action, **268,** 167
Geldzahler B. J. *see under* Cohen M. H., **268,** 405
Gerald P. *see under* Lubs H. A., **268,** 631
Gerschenfeld H. M. *see under* Piccolino M., **268,** 259
Gerschenfeld H. M. and Piccolino M. Muscarinic antagonists block cone to horizontal cell transmission in turtle retina, **268, 257**
Gesser H. D., Horsfall G. A., Gough K. M. and Krawchuk B. Transport of metal complex chlorides through plastic film membrane, **268,** 323
Gilbert L. I. *see under* Bollenbacher W. E., **268,** 660
Gillis S. and Smith K. A. Long term culture of tumour-specific cytotoxic T cells, **268,** 154
Gilpin E. A. *see under* Ondricek A., **268,** 374
Gilpin M. E. *see under* Ondricek A., **268,** 374
Gingell D. and Todd I. Long range attraction between red cells and hydrocarbon surface, **268,** 767
Gingell D., Todd I. and Parsegian V. A. Long range attraction between red cells and hydrocarbon surface, **268,** 767
Girling M. and Greig J. Palaeoecological investigations of the site at Hampstead Heath, London, **268,** 45
Girton J. R. *see under* Tiong S. Y., **268,** 435
Glynn I. I. *see under* Beaugé L. A., **268,** 355
Goertemiller C. C. Jr. *see under* Ellis R. A., **268,** 555
Goldanskii V. I. Mechanism for formaldehyde polymer formation in interstellar space, **268,** 612
Goodwin T. W. *see under* Britton G., **268,** 81
Gordon A. H. Amyloidosis, P component and complement, *news and views,* **268,** 14
Gospodarowicz D. *see under* Birdwell C. R., **268,** 528
Goto M., Tsuda Y. and Yatani A. Two mechanisms for positive inotropism of low-K Ringer solution in bullfrog *artrium,* **268,** 755
Gough K. M. *see under* Gesser H. D., **268,** 323
Green T. R. Significance of glucose oxidase in lignin degradation, **268,** 78
Greene L. A. *see under* Dichter M. A., **268,** 501
Greene L. A. and Rein G. Synthesis, storage and release of acetylcholine by a noradrenergic pheochromocytoma cell line, **268,** 349
Gregory A. H. *see under* Christensen I. P., **268,** 630
Greig J. *see under* Girling M., **268,** 45
Grinvald A., Salzberg B. M. and Cohen L. B. Simultaneous recording from several neurones in an invertebrate central nervous system, **268,** 140
Gschnait F. *see under* Stingl G., **268,** 245
Gubbins S. J. *see under* Jones M. D. R., **268,** 731
Gubenko I. *see under* Evgen'ev M., **268,** 766
Gupta A. P. *see under* Das Y. T., **268,** 139
Gurdon J. B. *see under* Wyllie A. H., **268,** 150
Guttenplan J. B. Inhibition of L-ascorbate of bacterial mutagenesis induced by two *N*-nitroso compounds, **268,** 368
Gutteridge W. E. *see under* Gaborak M., **268,** 339
Hada H. *see under* Konishi Y., **268,** 709
Hailwood E. A. *see under* Montadert L., **268,** 305
  see under Montadert L., **268,** 305
Hale G. *see under* Bates D. L., **268,** 313
Hale L. C. Particulate transport through the mesosphere and stratosphere, **268,** 710
Hall B. M., Roser B. J. and Dorsch S. E. Magnitude of memory to the major histocompatibility complex, **268,** 532
Hall Z. W. and Reiness C. G. Electrical stimulation of denervated muscles reduces incorporation of methionine into the ACh receptor, **268,** 655
Hallam A. Anoxic events in the Cretaceous ocean, *news and views,* **268,** 15
Hamada C. *see under* Hotta S., **268,** 733
Hammer P. R. *see under* Devlin J. C., **268,** 319
Hamp M. *see under* Lefkowitz R. J., **268,** 453
Hance A. J. and Crystal R. G. Rigid control of synthesis of collagen Types I and III by cells in culture, **268,** 152
Harris A. *see under* Soter S., **268,** 421
Harris A. J., Ziskind L. and Wigston D. Spontaneous release of transmitter from 'repressed' nerve terminals in axolotl muscle, **268,** 265
Harris J. M. Palaeomagnetic stratigraphy of the Koobi Fora Formation, east of Lake Turkana (Lake Rudolf), Kenya, *matters arising,* **268,** 669
Harris T. J. R. *see under* Sangar D. V., **268,** 648
  Terminal redundancy in avian oncornavirus RNA, **268,** 15
Harrison S. C. *see under* Earnshaw W. C., **268,** 598
Harrison W. *see under* Montadert L., **268,** 305
Harvey R. *see under* Pawson T., **268,** 416
Hasek M., Chutna J., Sladecek M. and Lodin Z. Immunological tolerance and tumour allografts in the brain, **268,** 68
Hashimoto M. *see under* Endo T., **268,** 74
Hashimoto T., Shimada H. and Sakai T. Thermoluminescence from gamma-ray irradiated normal paraffins, **268,** 225
Hatanaka K. *see under* Kitamura Y., **268,** 442
Hattersley P. M. *see under* Bailey J., **268,** 301
Haulet R., Zettwoog P. and Sabroux J. C. Sulphur dioxide discharge from Mount Etna, **268,** 715
Hauser D. *see under* Roemer D., **268,** 547
Haussler M. R. *see under* Hughes M. R., **268,** 347
Haydon D. A., Hendry B. M., Levinson S. R. and Requena J. The molecular mechanisms of anaesthesia, **268,** 356
Hayes P. H. *see under* Tiong S. Y., **268,** 435
Hayhoe F. G. J. *see under* Burns G. F., **268,** 243
Hecht A. D. Geomagnetism and climate, *matters arising,* **268,** 669
Hecht F. *see under* Lubs H. A., **268,** 631
Heertjes P. M. *see under* van Brakel J., **268,** 44
Heilbronn E. *see under* Mattsson H., **268,** 52
Heimer Y. M., Ben-Hur E. and Riklis E. Psoralen and near ultraviolet light: a probe for study of control of protein synthesis, **268,** 170
Hendry B. M. *see under* Haydon D. A., **268,** 356
Henneberry R. C., Smith C. C. and Tallman J. F. Relationship between beta-adrenergic receptors and adenylate cyclase in HeLa cells, **268,** 252
Heptonstall W. B. Plate linkage mechanism to account for oroclinal deformation in the Western Cordillera of North America, **268,** 271
Herberman R. B. *see under* Holden H. T., **268,** 250
Herring A. J. and Bevan E. A. Yeast virus-like particles possess a capsid-associated single-stranded RNA polymerase, **268,** 464
Hew C. L. *see under* Ananthanarayanan V. S., **268,** 560
Heyes L. M. *see under* Britton G., **268,** 81
Hicken A. and Irving E. Tectonic rotation in Western Canada, **268,** 219
Hill R. C. *see under* Roemer D., **268,** 547
Hillaire-Marcel C. *see under* Fairbridge R. W., **268,** 413
Hillhouse J. Palaeomagnetic stratigraphy of the Koobi Fora Formation, east of Lake Turkana (Lake Rudolf), Kenya — Reply, *matters arising,* **268,** 670
Hochstrasser R. M. Ramsey fringes in optical spectroscopy, *news and views,* **268,** 17
Hodgson P. E. Heavy ion transfer reactions, *news and views,* **268,** 18
  High-energy nucleus−nucleus collisions, *news and views,* **268,** 690
  Pion production in heavy-ion collisions, *news and views,* **268,** 586
Hoffman P. L. and Tabakoff B. Alterations in dopamine receptor sensitivity by chronic ethanol treatment, **268,** 551
Holden H. T. and Herberman R. B. Cytotoxicity against tumour-associated antigens not H−2 restricted, **268,** 250
Home J. M. *see under* Twibell J. D., **268,** 711
Hong J. *see under* Fratta W., **268,** 452
Hooper E. A. *see under* Bates D. L., **268,** 313
Hoor F. T. *see under* de Deckere E. A. M., **268,** 160
Hopwood D. A., Wright H. M., Bibb M. J. and Cohen S. N. Genetic recombination through protoplast fusion in *Streptomyces,* **268,** 171
Horsfall G. A. *see under* Gesser H. D., **268,** 323
Horwitz Z. D. *see under* Samuels H. H., **268,** 254
Hotta S., Kojima M., Fujisaka M., Uchida S., Kuroda H. and Hamada C. An antiviral substance extracted from *Streptococcus faecalis,* **268,** 733
Hovanessian A. G. *see under* Kerr I. M., **268,** 540
Hovanessian A. G., Brown R. E. and Kerr I. M. Synthesis of low molecular weight inhibitor of protein synthesis with enzyme from interferon-treated cells, **268,** 537
Howe R. F. *see under* Anderson J. R., **268,** 129
Hoyle F. and Wickramasinghe N. C. Polysaccharides and infrared spectra of galactic sources, **268,** 610
Hubbard W. B. Wave optics of the central spot in planetary occultations, **268,** 34
Hubbard W. B., Coyne G. V., Gehrels T., Smith B. A. and Zellner B. H. Observations of Uranus occultation events, **268,** 33
Hughes D. W. *see under* Brown J. C., **268,** 512
  Mass of Saturn's rings, *news and views,* **268,** 102
  The Star of Bethlehem — Reply, *matters arising,* **268,** 566
  Visiting Halley's Comet, *news and views,* **268,** 486
Hughes M. R., McCain T. A., Chang S. Y., Haussler M. R., Villareale M. and Wasserman R. H. Presence of 1,25-dihydroxyvitamin D_3-glycoside in the calcinogenic plant *Cestrum diurnum,* **268,** 347
Huguenin R. *see under* Roemer D., **268,** 547
Hull R. and Baker C. R. B. Forecasting in crop protection, *news and views,* **268,** 402
Hulse E. V. *see under* Edwards O., **268,** 565
Hunts S. and Oates K. Intracellular cationic counterion composition of an acid mucopolysaccharide, **268,** 370
Hutchinson R. Meteorite research old and new, *news and views,* **268,** 691
Hutt L. M. *see under* Lemon S. M., **268,** 268
Hutton J. T. Sodium and chloride in rainwater, *matters arising,* **268,** 568
Hydes D. J. Dissolved aluminium concentration in seawater, **268,** 136
Ihle J. N. *see under* Gardner M. B., **268,** 341
Ikushima T. Role of sister chromatid exchanges in chromatid aberration formation, **268,** 235
Illingworth A. J. *see under* Gaskell W., **268,** 124
Ingram V. M. *see under* Riggs M. G., **268,** 462
Irving E.see under Hicken A., **268,** 219
Isaacman R. NP0532 and a hole in the Crab Negula, **268,** 317
Isaacs N. S., Javaid K. and Rannala E. Pressure effects on proton tunnelling, **268,** 372
Ishigami T. *see under* Endo T., **268,** 74
Iversen L. L. *see under* Jessell T. M., **268,** 549
  see under Reubi J.-C., **268,** 652
Iversen S. D. *see under* Tye N. C., **268,** 741
Iwatsuki N. and Petersen O. H. Acetylcholine-like effects of intracellular calcium application in pancreatic acinar cells, **268,** 147
Iwazumi T. *see under* Pollack G. H., **268,** 757
Jaffe R. L. Quark confinement, *review article,* **268,** 201
Jakobisiak M. *see under* Kaminski M., **268,** 238
Javaid K. *see under* Isaacs N. S., **268,** 372
Jenkins D. G. Atlantic palaeotemperatures during the Cainozoic, *matters arising,* **268,** 373
Jernigan G. *see under* Rappaport S., **268,** 705
Jessell T. M. *see under* Reubi J.-C., **268,** 652
Jessell T. M. and Iversen L. L. Opiate analgesics inhibit substance P release from rat trigeminal nucleus, **268,** 549
Johnson K. A. *see under* Tyrer N. M., **268,** 759
Johnson R. D. *see under* Bartell L. S., **268,** 707
Johnson-Laird P. N. *see under* Wykes T., **268,** 326
Johnston G. A. R. *see under* Krogsgaard-Larsen P., **268,** 53
Jones K. W. Snake's eye view of Adam and Eve, *news and views,* **268,** 107
Jones M. D. R. and Gubbins S. J. Modification of circadian flight activity in the mosquito *Anopheles gambiae* after insemination, **268,** 731
Jukes T. H. Berkeley women, *news,* **268,** 582

COSPAR in Israel, *news*, **268,** 293
Julian P. R. *see under* Levanon N., **268,** 514
Junkerman W. *see under* Roedel W., **268,** 320
Kagami H. *see under* Montadert L., **268,** 305
Kajii T. and Ohama KM Androgenetic origin of hydatidiform mole, **268,** 633
Kaminska G. *see under* Kaminski M., **268,** 238
Kaminski M., Kaminska G., Jakobisiak M. and Brzezinski W. Inhibition of lymphocyte-induced angiogenesis by isolated chondrocytes, **268,** 238
Kaneoka I. and Ozima M. ^{40}Ar/^{39}Ar ages of a diabase sill and a basalt in the Central-Pacific Basin, **268,** 132
Karlsson H. R. *see under* Kristjansson L., **268,** 325
Karplus M. *see under McCammon J. A.*, **268,** 765
Kasarjian J. *see under* Aguayo A. J., **268,** 753
Kaufman J. F. *see under* Springer T. A., **268,** 213
Kawade Y. *see under* Sokawa Y., **268,** 236
Kawakami T. G., Sun L. and McDowell T. S. Infectious primate type-C virus shed by healthy gibbons, **268,** 448
Kay R. F. Diets of early Miocene African hominoids, **268,** 628
Kellermann K. I. *see under* Cohen M. H., **268,** 405
Kellett D. N. *see under* Marsh N. A., **268,** 726
Kelly P. M. Volcanic dust veils and North Atlantic climatic change, **268,** 616
Kelus A. S. *see under* Taussig M. J., **268,** 178
Kennedy B. G. and De Weer P. Relationship between Na : K and Na : Na exchange by the sodium pump of skeletal muscle, **268,** 165
Kerr I. M. *see under* Hovanessian A. G., **268,** 537
Kerr I. M., Brown R. E. and Hovanessian A. G. Nature of inhibitor of cell-free protein synthesis formed in response to interferon and double-stranded RNA, **268,** 540
Kershaw S. *see under* Riding R., **268,** 178
Kimberling W. J. *see under* Lubs H. A., **268,** 631
Kindred B. and Corley R. B. A T cell-replacing factor specific for histocompatibility antigens in mice, **268,** 531
Kinosita K. Jr and Tsong T. Y. Formation and resealing of pores of controlled sizes in human erythrocyte membrane, **268,** 438
Kiss G. B. *see under* Kondorosi A., **268,** 525
Kitamura S. *see under* Sumino K., **268,** 73
Kitamura Y., Shimada M., Hatanaka K. and Miyano Y. Development of mast cells from grafted bone marrow cells in irradiated mice, **268,** 442
Klareskog L., Tjernlund U. M., Forsum U. and Peterson P. A. Epidermal Langerhans cells express Ia antigens, **268,** 248
Kledzik G. S. *see under* Cusan L., **268,** 544
Klein C. and Darmon M. Effects of cyclic AMP pulses on adenylate cyclase and the phosphodiesterase inhibitor of *D. discoideum*, **268,** 76
Klimpel G. R. *see under* Byus C. V., **268,** 63
Knapp W. *see under* Stingl G., **268,** 245
Koch Y. *see under* Okon E., **268,** 445
Kojima M. *see under* Hotta S., **268,** 733
Kondorosi A., Kiss G. B., Forrai T., Vincze E. and Banfalvi Z. Circular linkage map of *Rhizobium meliloti* chromosome, **268,** 525
Kongshavn P. *see under* Aguayo A. J., **268,** 753
Koopman B. L. *see under* Benemann J. R., **268,** 19
Korner M. *see under* Schramm M., **268,** 310
Kornishi Y., Saijo H., Hada H. and Tamura M. Formation of silver mirror by photolysis of thin films of high molecular silver compounds, **268,** 709
Kors K. *see under* Kristjansson L., **268,** 325
Krankowsky D. *see under* Arnold F., **268,** 218
Krawchuk B. *see under* Gesser H. D., **268,** 323
Krebs J. Optimal foraging: theory and experiment, *news and views*, **268,** 583
Krienen F. *see under* Bailey J., **268,** 301
Kristjansson L., Thors K. and Karlsson H. R. Confirmation of central volcanoes off the Icelandic coast, **268,** 325
Krogsgaard-Larsen P., Johnston G. A. R., Lodge D. and Curtis D. R. A new class of GABA agonist, **268,** 53
Kung C. *see under* Oertel D., **268,** 120
Kung L. S. *see under* Banerjee S. P., **268,** 455
Kurki P., Linder E., Virtanen I. and Stenman S. Human smooth muscle autoantibodies reacting with intermediate (100 Å) filaments, **268,** 240
Kuroda H. *see under* Hotta S., **268,** 733
Kuwahara A. *see under* Endo T., **268,** 74
Läuger P. Ion transport and rotation of bacterial flagella, **268,** 360
Labrie F. *see under* Cusan L., **268,** 544
Laegaard S. *see under* Søondergaard M., **268,** 232
Lamb C. J. and Dudley R. K. More light on enzyme control, *news and views*, **268,** 692
Lambert D. H., Spannbauer P. M. and Parsons R. L. Densensitisation does not selectively alter sodium channels, **268,** 553
Landon J. Enzymoimmunoassay: techniques and uses, *news and views*, **268,** 483
Lang F. Synaptic and septate neuromuscular junctions in embryonic lobster muscle, **268,** 458
Lange F. *see under* Bailey J., **268,** 301
Larsen B. *see under* Arnašson A., **268,** 527
Larson R. G., Scriven L. E. and Davis H. T. Percolation theory of residual phases in porous media, **268,** 409
Larsson P.-O. *see under* Mattiasson B., **268,** 519
Latham J. *see under* Gaskell W., **268,** 124
Lauder J. M. and Mugnaini E. Early hyperthyroidism alters the distribution of mossy fibres in the rat hippocampus, **268,** 335
Lawton J. H. *see under* Pimm S. L., **268,** 329
Leeder M. R. and Zeidan R. Giant late Jurassic sabkhas of Arabian Tethys, **268,** 42
Lefkowitz R. J. and Hamp M. Comparison of specificity of agonist and antagonist radioligand binding to beta-adrenergic receptors, **268,** 453
Leidner L. *see under* Roedel W., **268,** 320
Lemon S. M., Hutt L. M., Shaw J. E., Li J.-L. H. and Pagano J. S. Replication of EBV in epithelial cells during infectious mononucleosis, **268,** 268
Lesley J. F. and Lennon V. A. Transitory expression of Thy-1 antigen in skeletal muscle development, **268,** 163
Levanon N., Julian P. R. and Suomi V. E. Antarctic topography from balloons, **268,** 514
Levine A. *see under* Evgen'ev M., **268,** 766
Levine L. Chemical carcinogens stimulate canine kidney (MDCK) cells to produce prostaglandins, **268,** 447
Levinson S. R. *see under* Haydon D. A., **268,** 356
Levit S. *see under* Brostoff S. W., **268,** 752
Levy J. A. *see under* Gardner M. B., **268,** 341
Lewis K. E. and O'Day D. H. Sex hormone of *Dictyostelium discoideum* is volatile, **268,** 730
Lewis M. G. *see under* Rowden G., **268,** 247
Li F. *see under* Rappaport S., **268,** 705
Li J.-L. H. *see under* Lemon S. M., **268,** 268
Lindberg S. E. and Turner R. R. Mercury emissions from chlorine-production solid waste deposits, **268,** 133
Linder E. *see under* Kurki P., **268,** 240
Linfield R. P. *see under* Cohen M. H., **268,** 405
Llorens-Cortes C. *see under* Pollard H., **268,** 745
Lockley W. J. S. *see under* Britton G., **268,** 81
Lodge D. *see under* Korgsgaard-Larsen P., **268,** 53
Lodin Z. *see under* Hasek M., **268,** 68
London W. T. Epizootiology, transmission and approach to prevention of fatal simian haemorrhagic fever in rhesus monkeys, **268,** 344
Long A. R. Amorphous semiconductors, *news and views*, **268,** 399
Lopez A. F. *see under* Sanderson C. J., **268,** 340
Loubet M. *see under* Flynn G. J., **268,** 717
Loyd R. M. *see under* Tassava R. A., **268,** 49
Lubs H. A., Kimberling W. J., Hecht F., Patil S. R., Brown J., Gerald P. and Summitt R. L. Racial differences in the frequency of Q and C chromosomal heteromorphisms, **268,** 631
Lucas D. O. *see under* Byus C. V., **268,** 63
Lumsden D. N. *see under* Montadert L., **268,** 305
Lunde G. and Bjorseth A. Polycyclic aromatic hydrocarbons in long-range transported aerosols, **268,** 518
Lyne A. G. and Ritchings R. T. The binary pulsar observed at four radio frequencies, **268,** 606
Lyttle C. R. and DeSombre E. R. Generality of oestrogen stimulation of peroxidase activity in growth-response tissues, **268,** 337
Lyubarskiy A. L. *see under* Fesenko E. E., **268,** 562
Mackintosh P. *see under* Arnason A., **268,** 527
Macnair M. R. Major genes for copper tolerance in *Mimulus guttatus*, **268,** 428
Mahy B. W. J. Reading the tumour virus genome, *news and views*, **268,** 398
Malcolm A. D. B. Binding the *lac* repressor, **268,** 196
Maráz A. *see under* Ferenczy L., **268,** 524
Marsh N. A., Clarke C. A., Rothschild M. and Kellett D. N. *Hypolimnas bolina* (L.) a mimic of danaid butterflies and its model *Euploea core* (Cram.) store cardioactive substances, **268,** 726
Marshall N. J., von Borcke S., Florin-Christensen A. and Ekins R. P. Propranolol increases binding of thyrotropin to thyroid membranes, **268,** 58
Marshall W. H. *see under* Arnason A., **268,** 527
Martin E. S. *see under* Stevens R. A., **268,** 364
Martin G. R. Absolute visual threshold and scotopic spectral sensitivity in the tawny owl *Strix aluco*, **268,** 636
Masters P. M., Bada J. L. and Zigler J. S. Aspartic acid racemisation in the human lens during ageing and in cataract formation, **267,** 71
Matsui T. *see under* Prasad C., **268,** 142
Matthews R. K. *see under* Shackleton N. J., **268,** 618
Mattiasson B., Larsson P.-O. and Mosbach K. The microbe thermistor, **268,** 519
Mattsson H., Brandt K. and Heilbronn E. Bicyclic phosphorus esters increase the cyclic GMP level in rat cerebellum, **268,** 52
May R. M. Caves as islands, *news and views*, **268,** 589
Elephants in Uganda, **268,** 196
Population genetics and cultural inheritance, *news and views*, **268,** 11
McCain T. A. *see under* Hughes M. R., **268,** 347
McCammon J. A. and Karplus M. Internal motions of antibody molecules, **268,** 765
McClellan G. B. and Winegrad S. Membrane control of cardiac contractility, **268,** 261
McDowell T. S. *see under* Kawakami T. G., **268,** 448
McIntosh A. T. *see under* Russell S. W., **268,** 69
Mészáros P., Meyer F. and Pringle J. E. Cyg X-3 gamma rays from a magnetic accretion disk, **268,** 420
Medvedev Z. Soviet genetics: new controversy, *news*, **268,** 285
Meier C. and Bischoff A. Dysmyelination in Jimpy mouse due to astroglial hyperlasia? *matters arising*, **268,** 177
Mellanby K. Acid rain, *news*, **268,** 99
Frugal farming, *news*, **268,** 686
People projections, *news*, **268,** 395
The biter bit, *news*, **268,** 10
Mendlewicz J. and Rainer J. D. Adoption study supporting genetic transmission in manic-depressive illness, **268,** 327
Merigan T. C., Oldstone M. B. A. and Welsh R. M. Interferon production during lymphocytic choriomeningitis virus infection of nude and normal mice, **268,** 67
Meyer F. *see under* Mészáros P., **268,** 420
Migeon B. R. *see under* Corsaro C. M., **268,** 737
Miledi R. *see under* Anderson C. R., **268,** 663
Miledi R., Parker I. and Schalow G. Calcium transients in frog slow muscle fibres, **268,** 750
Mills A. A. Dust clouds and frictional generation of glow discharges on Mars, **268,** 614
Milner A. D. and Dunne J. J. Lateralised perception of bilateral chimaeric faces by normal subjects, **268,** 175
Milner S. M. Activation of mouse spleen cells by a single short pulse of mitogen, **268,** 441
Mitchison N. A. Neoplastic transformation, *news and views*, **268,** 106
Miyano Y. *see under* Kitamura Y., **268,** 442
Moffet A. T. *see under* Cohen M. H., **268,** 405
Molday R. S., Yen S. P. S. and Rembaum A. Application of magnetic microspheres in labelling and separation of cells, **268,** 437
Mollon J. D. The oddity of blue, *news and views*, **268,** 587
Montadert L., Roberts D. G., Auffret G. A., Bock W., DuPeuble P. A., Hailwood E. A., Harrison W., Kagami H., Lumsden D. N., Muller C., Schnitker D., Thompson R. W., Thompson T. L. and Timofeev P. P. Rifting and subsidence on passive continental margins in the North East Atlantic, **268,** 305
Moorbath S. *see under* Chapman H. J., **268,** 41
Moore C. B. *see under* Gaskell W., **268,** 124
Moore P. D.. Ancient distribution of lime trees in Britain, *news and views*, **268,** 13
Moore P. D. Atmospheric carbon dioxide and forest clearance, *news and views*, **268,** 296
Science in the Himalayas, *news*, **268,** 682
Morgan D. New quarks . . . now the good news, *news and views*, **268,** 297
Mosbach K. *see under* Mattiasson B., **268,** 519
Moss D. J., Scott W. and Pope J. H. An immunological basis for inhibition of transformation of human lymphocytes by EB virus, **268,** 735
Mugnaini E. *see under* Lauder J. M., **268,** 335
Muller C. *see under* Montadert L., **268,** 305
murata T. *see under* Endo T., **268,** 74
Murlis J. and Bettany B. W. Night flight towards a sex pheromone source by male *Spodoptera littoralis* (Boisd.) (lepidoptera, Noctuidae), **268,** 433
Murthy B. S. *see under* Deshpande M. R., **268,** 614
Myatt L. and Elder M. G. Inhibition of platelet aggregation by a placental substance with prostacyclin-like activity, **268,** 159
Nardi J. B. Are cell affinity relationships for insect epidermal tissues evidence for combinatorial control? **268,** 665
Nash A. A., *see under* Burns G. F., **268,** 243
Neidle S. Approaches to effective anti-cancer drugs, *news and views*, **268,** 195
Neumann J. R. *see under* Riggs M. G., **268,** 462
Nevers P. and Saedler H. Transposable genetic dlements as agents of gene instability and chromosomal rearrangements, *review article*, **268,** 109
Nevo E. *see under* Brown A., **268,** 430
Nickla H. Maternal effects determine effective lethal phase of *carnation-light* synthetic lethal in *Drosophila melanogaster*, **268,** 638
Nicolson G. L. *see under* Birdwell C. R., **268,** 528
Nomoto A., Detjen B., Pozzatti R. and Wimmer E. The location of the polio genome protein in viral RNAs and its implication for RNA synthesis, **268,** 208
Norenburg M. D. *see under* Riepe R. E., **268,** 654
Norman C. OTA's stormy ride, *news*, **268,** 4
Northmore D. P. M. Spatial summation and light adaptation in the goldfish visual system, **268,** 450
Nottale L. and Vigier J. P. Continuous increase of Hubble modulus behind clusters of galaxies, **268,** 608
Nowlin W. D. Jr. *see under* Baker D. J. Jr, **268,** 696
Nugteren D. H. *see under* De Deckere E. A. M., **268,** 160
Nurse K. *see under* Thompson E. J., **268,** 241
Nwuke E. I. *see under* Solomons C. C., **268,** 55
Oates K. *see under* Hunt S., **268,** 370
Oatley S. J. *see under* Blake C. C. F., **268,** 115
O'Day D. H. *see under* Lewis K. E., **268,** 730
Oertel D., Schein S. J. and Kung C. Separation of membrane currents using a *Paramecium* mutant, **268,** 120
Ohama K. *see under* Kajii T., **268,** 633
Ohashi O. *see under* Takewaki T., **268,** 749
Ohishi N. *see under* Fujihira M., **268,** 226
Okon E. and Koch Y. Localisation of gonadotropin-releasing hormone in the circumventricular organs of human brain, **268,** 445
Olaisen B. *see under* Arnason A., **268,** 527
Oldstone M. B. A. *see under* Merigan T. C., **268,** 67
Olianas M. *see under* Di Chiara G., **268,** 743
Ondricek A., Gilpin E. A., Soule M. E. and Gilpin M. E. U-shaped gene frequency distributions — Reply, *matters arising*, **268,** 374
Orly J. *see under* Schramm M., **268,** 310
Osa T. *see under* Fujihira M., **268,** 226
Oswald W. J. *see under* Benemann J. R., **268,** 19
Pagano J. S. *see under* Lemon S. M., **268,** 268
Palmer T. J. *see under* Edwards O., **268,** 565
Pankhurst R. J. Open system crystal fractionation and incompatible element variation in basalts, **268,** 36
Pansky B. *see under* Senitzer D., **268,** 158
Parker I. *see under* Miledi R., **268,** 750
Parsegian V. A. *see under* Gingell D., **268,** 767
Parshad R. and Sanford K. K. Intermittent exposure to

fluorescent light extends lifespan of human diploid fibroblasts in culture, **268,** 736

Parsons R. L. *see under* Lambert D. H., **268,** 553

Paterson M. P. Sodium and chloride in rainwater — Reply, *matters arising,* **268,** 568

Patil S. R. *see under* Lubs H. A., **268,** 631

Pauliny-Toth I. I. K. *see under* Cohen M. H., **268,** 405

Paulsen D. F. and Finch R. A. Age- and region-dependent concavalin A reactivity of chick wing-bud mesoderm cells, **268,** 639

Pawson T., Harvey R. and Smith A. E. The size of Rous sarcoma virus mRNAs active in cell-free translation, **268,** 416

Pemberton J. M. and Fisher P. R. 2,4-D plasmids and persistence, **268,** 732

Penkett S. A. *see under* Brice K. A., **268,** 127

perham R. N. *see under* Bates D. L., **268,** 313

Peterkofsky A. *see under* Prasad C., **268,** 142

Petersen O. H. *see under* Iwatsuki N., **268,** 147

Peterson P. A. *see under* Klareskog L., **268,** 248

Pettersson R. F., Flanegan J. B., Rose J. K. and Baltimore D. 5″-Terminal nucleotide sequences of polio virus polyribosomal RNA and virion RNA are identical, **268,** 270

Peucat J. J. and Cogne J. Geochronology of some blueschists from Ile de Groix, France, **268,** 131

Phillips J. M. and Waldmann H. Monogamous T helper cell, **268,** 641

Picasso E. *see under* Bailey J., **268,** 301

Piccolino M. and Gerschenfeld H. M. Lateral interactions in the outer plexiform layer of turtle retinas after atropine block of horizontal cells, **268,** 259

Pichler W. J. *see under* Stingl G., **268,** 245

Pickersgill B. Taxonomy and the origin and evolution of cultivated plants in the New World, *review article,* **268,** 591

Pillarisetty R. J. *see under* Gardner M. B., **268,** 341

Pillsbury R. D. *see under* Baker D. J. Jr, **268,** 696

Pimm S. L. and Lawton J. H. Number of trophic levels in ecological communities, **268,** 329

Pless J. *see under* Roemer D., **268,** 547

Pockley P. When two academies meet, *news,* **268,** 187

Pollack G. H., Iwazumi T., ter Keurs H. E. D. J. and Shibata E. F. Sarcomere shortening in striated muscle occurs in stepwise fashion, **268,** 757

Pollard H., Llorens-Cortes C. and Schwartz J. C. Enkephalin receptors on dopaminergic neurones in rat striatum, **268,** 745

Pope J. H. *see under* Moss D. J., **268,** 735

Potts R., Fournier M. J. and Ford N. C. Effect of aminoacylation on the conformation of yeast phenylalanine tRNA, **268,** 563

Powers J. M. *see under* Brostoff S. W., **268,** 752

Powers M. L. *see under* Villee D. B., **268,** 156

Powls R. *see under* Britton G., **268,** 81

Pozzatti R. *see under* Nomoto A., **268,** 208

Prasad C., Matsui T. and Peterkofsky A. Antagonism of ethanol narcosis by histidyl-proline diketopiperazine, **268,** 142

Pratt G. E. *see under* Tobe S. S., **268,** 728

Press F. *see under* Briggs P. L., **268,** 125

Preuss E. *see under* Cohen M. H., **268,** 405

Price J. *see under* Wyllie A. H., **268,** 150

Pringle J. The puzzles of Hercules, *news and views,* **268,** 484

Pringle J. E. *see under* Mészáros P., **268,** 420

Prives J. and Shinitzky M. Increased membrane fluidity precedes fusion of muscle cells, **268,** 761

Quétier F. and Vedel F. Heterogeneous population of mitochondrial DNA molecules in higher plants, **268,** 365

Quenby J. J. *see under* Coe M. J., **268,** 508

Cosmic ray age, soft X-rays and the galactic wind, *news and views,* **268,** 401

Rahn K. A., Borys R. D. and Shaw G. E. The Asian source of Arctic haze bands, **268,** 713

Rainer J. D. *see under* Mendlewicz J., **268,** 327

Ramachandran V. S., Clarke P. G. H. and Whitteridge D. Cells selective to binocular disparity in the cortex of newborn lambs, **268,** 333

Rannala E. *see under* Isaacs N. S., **268,** 372

Rappaport S., Clark G. W., Dower R., Doxsey R., Jernigan G. and Li F. Variable mid-latitude X-ray source 3U0042+32, **268,** 705

Rastogi R. G. Geomagnetic storms and electric fields in the equatorial ionosphere, **268,** 422

Redfearn J. ILL at ease, *news,* **268,** 287

Rein G. *see under* Greene L. A., **268,** 349

Reiness C. G. *see under* Hall Z. W., **268,** 655

Rembaum A. *see under* Molday R. S., **268,** 437

Remington J. S. *see under* Wing E. J., **268,** 642

Requena J. *see under* Haydon D. A., **268,** 356

Reubi J.-C., Iversen L. L. and Jessell T. M. Dopamine selectively increases ^{3}H-GABA release from slices of rat substantia nigra *in vitro,* **268,** 652

Rice M. J. Blowfly ovipositor receptor neurone sensitive to monovalent cation concentration, **268,** 747

Rich V. Heading for Honolulu, *news,* **268,** 578

Richards E. G. Ribosomal switches, *news and views,* **268,** 585

Richardson J. S. Beta-Sheet topology and the relatedness of proteins, **268,** 495

Riding R. and Kershaw S. Nature of stromatoporoids, *matters arising,* **268,** 178

Riepe R. E. and Norenburg M. D. Müller cell localisation of glutamine synthetase in rat retina, **268,** 654

Rigby P. W. J. Insulin genes into *E. coli:* first step from fantasy to fact, **268,** 198

Riggi S. J. *see under* Banerjee S. P., **268,** 455

Riggs M. G., Whittaker R. G., Neumann J. R. and Ingram V. M. *n*-Butyrate causes histone modification in He-La and Friend erythroleukaemia cells, **268,** 462

Riklis E. *see under* Heimer Y. M., **268,** 170

Ringel S. P. *see under* Solomons C. C., **268,** 55

Ritchings R. T. *see under* Lyne A. G., **268,** 606

Roberts D. G. *see under* Montadert L., **268,** 305

Roberts E. H. and Ellis R. H. Prediction of seed longevity at sub-zero temperatures and genetic resources conservation, **268,** 431

Robinson J. C. *see under* Chou J. Y., **268,** 543

Roedel W., Junkerman W. and Leidner L. Radioactive ^{35}S in an atmospheric SO$_2$ and SO$_2$ oxidation rate measurement, **268,** 320

Roemer D., Buescher H. H., Hill R. C., Pless J., Bauer W., Cardinaux F., Closse A., Hauser D. and Huguenin R. A synthetic enkephalin analogue with prolonged parenteral and oral analgesic activity, **268,** 547

Romano G. *see under* Barbieri C., **268,** 318

Romans J. C. C. Stratigraphy of buried soil at Teindland Forest, Scotland, **268,** 622

Romney J. D. *see under* Cohen M. H., **268,** 405

Rose G. D. and Wetlaufer D. B. The number of turns in globular proteins, **268,** 769

Rose J. K. *see under* Pettersson R. F., **268,** 270

Roser B. J. *see under* Hall B. M., **268,** 532

Ross L. J. N. Antiviral T cell-mediated immunity in Marek's disease, **268,** 644

Rothschild M. *see under* Marsh N. A., **268,** 726

Rowden G., Lewis M. G. and Sullivan A. K. Ia antigen expression on human epidermal Langerhans cells, **268,** 247

Rowlands D. J. *see under* Sangar D. V., **268,** 648

Roxburgh I. W. Large number hypothesis and continuous creation cosmologies, **268,** 504

Rush H. *see under* Blythe C., **268,** 386

Russell D. H. *see under* Byus C. V., **268,** 63

Russell M. A. *see under* Tiong S. Y., **268,** 435

Russell S. W. and McIntosh A. T. Macrophages isolated from regressing Moloney sarcomas are more cytotoxic than those recovered from progressing sarcomas, **268,** 69

Ryning F. W. *see under* Wing E. J., **268,** 642

Sabroux J. C. *see under* Haulet R., **268,** 715

Saedler H. *see under* Nevers P., **268,** 109

Saijo H. *see under* Konishi Y., **268,** 709

Sakai T. *see under* Hashimoto T., **268,** 225

Salzberg B. M. *see under* Grinvald A., **268,** 140

Sambrook J. Adenovirus amazes at Cold Spring Harbor, *news and views,* **268,** 101

Samuels H. H., Horwitz Z. D., Stanley F., Casanova J. and Shapiro L. E. Thyroid hormone controls glucocorticoid action in cultured GH$_1$ cells, **268,** 254

Sanderson C. J., Lopez A. F. and Bunn Moreno M. M. Eosinophils and not lymphoid K cells kill *Trypanosoma cruzi* epimastigotes, **268,** 340

Sandoe C. P. *see under* Wira C. R., **268,** 534

Sanford K. L. *see under* Parshad R., **268,** 736

Sangar D. V., Rowlands D. J., Harris T. J. R. and Brown F. Protein covalently linked to foot-and-mouse disease virus RNA, **268,** 648

Sargent P. B. and Dennis M. J. Formation of synapses between parasympathetic neurones deprived of preganglionic innervation, **268,** 456

Saxena R. K. and Talwar G. P. An anterior pituitary factor stimulates thymidine incorporation in isolated thymocytes, **268,** 57

Schally A. V. *see under* Cusan L., **268,** 544

Schalow G. *see under* Miledi R., **268,** 750

Scheffler I. E. *see under* Ditta G., **268,** 64

Schein S. J. *see under* Oertel D., **268,** 120

Schilizzi R. T. *see under* Cohen M. H., **268,** 405

Schnitker D. *see under* Montadert L., **268,** 305

Schove D. J. Maya dates AD 352-1296, *matters arising,* **268,** 670

Schramm M., Orly J., Eimerl S. and Korner M. Coupling of hormone receptors to adenylate cyclase of different cells by cell fusion, **268,** 310

Schwartz J. C. *see under* Pollard H., **268,** 745

Scordilis S. P. and Adelstein R. S. Myoblast myosin phosphorylation is a prerequisite for actin activation, **268,** 558

Scott W. *see under* Moss D. J., **268,** 735

Scriven L. E. *see under* Larson R. G., **268,** 409

Scrutton R. A. *see under* Dingle R. V., **268,** 720

Secher D. S. Multispecific antibodies, *news and views,* **268,** 689

Seilstad H. A. *see under* Cohen M. H., **268,** 405

Senanayake F. R. Habitat values and endemicity in the vanishing rain forests of Sri Lanka, *matters arising,* **268,** 568

Senitzer D., Cafruny W., Pansky B. and Freimer E. H. Spontaneous and induced cell-mediated reactivity to syngeneic cells, **268,** 158

Sethia G. *see under* Deshpande M. R., **268,** 614

Setlow J. K. The shape of the ultraviolet inactivation curve for transforming DNA, **268,** 169

Shackleton N. J. and Matthews R. K. Oxygen isotope stratigraphy of Late Pleistocene coral terraces in Barbados, **268,** 618

Shaffer D. B. *see under* Cohen M. H., **268,** 405

Shapiro L. E. *see under* Samuels H. H., **268,** 254

Sharkey A. G. *see under* White C. M., **268,** 620

Shaw G. E. *see under* Rahn K. A., **268,** 713

Shaw J. E. *see under* Lemon S. M., **268,** 268

Sherwell C. Man-made process, *news,* **268,** 478

Shibata E. F. *see under* Pollack G. H., **268,** 757

Shimada H. *see under* Hashimoto T., **268,** 225

Shimada M. *see under* Kitamura Y., **268,** 442

Shinitzky M. *see under* Prives J., **268,** 761

Shultz J. L. *see under* White C. M., **268,** 620

Shuman H. *see under* Somlyo A. V., **268,** 556

Skamene E. *see under* Aguayo A. J., **268,** 753

Skirball L. R., Braizer L. and Dretchen K. L. Evidence for a cyclic nucleotide-mediated calcium flux in motor nerve terminals, **268,** 352

Skoff R. P. Dysmyelination in Jimpy mouse due to astroglial hyperlasia? ¼ Reply, *matters arising,* **268,** 177

Sladecek M. *see under* Hasek M., **268,** 68

Slegers J. F. G. *see under* Augustus J., **268,** 657

Smalley I. J. The Warsaw-Moscow soil structure project, *news and views,* **268,** 487

Smith A. E. *see under* Pawson T., **268,** 416

Smith B. A. *see under* Hubbard W. B., **268,** 33

Uranus rings: an optical search, **268,** 32

Smith C. C. *see under* Henneberry R. C., **268,** 252

Smith J. M. Why the genome does not congeal, *review article,* **268,** 693

Smith P. J. Evidence for Earth expansion? *news and views,* **268,** 200

Isotherm limits ocean magnetism, *news and views,* **268,** 299

New shocks in northeastern Alaska, *news and views,* **268,** 12

New sulphide mineral discovered, *news and views,* **268,** 585

The return of whole-mantle convection, *news and views,* **268,** 687

Smith S. J. Calcium and muscle regulation, *news and views,* **268,** 400

Smith S. L. *see under* Bollenbacher W. E., **268,** 660

Søndergaard M. and Laegaard S. Vesicular-arbuscular mycorrhiza in some aquatic vascular plants, **268,** 232

Soderberg K. *see under* Ditta G., **268,** 64

Sokawa Y., Watanabe Yoshihiko, Watamabe Yuji and Kawade Y. Interferon suppresses the transition of quiescent 3T3 cells to a growing state, **268,** 236

Solomons C. C., Ringel S. P., Nwuke E. I. and Suga H. Abnormal adenine metabolism of erythrocytes in Duchenne and myotonic muscular dystrophy, **268,** 55

Somlyo A. P. *see under* Somlyo A. V., **268,** 556

Somlyo A. V., Shuman H. and Somlyo A. P. Composition of sarcoplasmic reticulum *in situ* by electron probe X-ray microanalysis, **268,** 556

Soter S. and Harris A. Are striations on Phobos evidence for tidal stress? **268,** 421

Soule M. E. *see under* Ondricek A., **268,** 374

Spannbauer P. M. *see under* Lambert D. H., **268,** 553

Spano P. F. *see under* Di Chiara G., **268,** 743

Spooner L. L. R. and Barry R. D. Participation of DNA-dependent RNA polymerase II in replication of influenza viruses, **268,** 650

Springer T. A., Kaufman J. F., Terhorst C. and Strominger J. L. Purification and structural characterisation of human *HLA*-linked B-cell antigens, **268,** 213

Stacey F. D. *see under* Brennan B. J., **268,** 220

Stanley F. *see under* Samuels H. H., **268,** 254

Stanworth C. W. *see under* Badham J. P. N., **268,** 516

Stapleton R. P. Carboniferous unconformity in southern Africa, **268,** 222

Stenman S. *see under* Kurki P., **268,** 240

Stetson D. L. *see under* Ellis R. A., **268,** 555

Stevens R. A. and Martin E. S. Ion-adsorbent substomatal structures in *Tradescantia pallidus,* **268,** 364

Stevenson D. J. Hydrogen in the Earth's core, **268,** 130

Stingl G., Wolff-Schreiner E. C., Pichler W. J., Gschnait F., Knapp W. and wolff K. Epidermal Langerhans cells bear Fc and C3 receptors, **268,** 245

Strominger J. L. *see under* Springer T. A., **268,** 213

Suga H. *see under* Solomons C. C., **268,** 55

Sullivan A. K. *see under* Rowden G., **268,** 247

Sumino K., Yamamoto R. and Kitamura S. A role of selenium against methylmercury toxicity, **268,** 73

Summitt R. L. *see under* Lubs H. A., **268,** 631

Sun L. *see under* Kawakami T. G., **268,** 448

Sundharadas G. Do antigen-specific helper factors in rabbit lack V-region Ig determinants? *matters arising,* **268,** 178

Suomi V. E. *see under* Levanon N., **268,** 514

Tabakoff B. *see under* Hoffman P. L., **268,** 551

Tagliamonte A. *see under* Di Chiara G., **268,** 743

Takewaki T. and Ohashi O. Non-cholinergic excitatory transmission to intestinal smooth muscle cells, **268,** 749

Talal N. *see under* Gardner M. B., **268,** 341

Talbot M. R. and Delibrias G. Holocene variations in the level of Lake Bosumtwi, Ghana, **268,** 722

Tallman J. F. *see under* Henneberry R. C., **268,** 252

Talwar G. P. *see under* Saxena R. K., **268,** 57

Tamura M. *see under* Konishi Y., **268,** 709

Tasai H. *see under* Endo T., **268,** 74

Tassava R. A. and Loyd R. M. Injury requirement for initiation of regeneration of newt limbs which have whole skin grafts, **268,** 49

Tauber C. A. and Tauber M. J. A genetic model for sympatric speciation through habitat diversification and seasonal isolation, **268,** 702

Tauber M. J. *see under* Tauber C. A., **268,** 702

Taussig M. J., Finch A. P. and Kelus A. S. Do antigen-specific helper factors in rabbit lack V-region Ig determinants? — Reply, *matters arising,* **268,** 178

teisberg P. *see under* Arnason A., **268,** 527

Tenaza R. R. and Tilson R. L. Evolution of long-distance alarm calls in Kloss's gibbon, **268,** 233

Teng M-H., Bartholomew J. C. and Bissell M. J. Synergism between anti-microtubule agents and growth stimulants in enhancement of cell cycle traverse, **268,** 739

ter Keurs H. E. D. J. *see under* Pollack G. H., **268,** 757

Terhorst C. *see under* Springer T. A., **268,** 213

Thomas C. Making paper work, *news,* **268,** 188

Thompson E. J., Yasin R., Beers G. V., Nurse K. and Al-Ani S. Myogenic defects in human muscular dystrophy, **268,** 241
Thompson R. W. *see under* Montadert L., **268,** 305
Thompson T. L. *see under* Montadert L., **268,** 305
Thorne H. V. and Dermott E. Y-forms as possible intermediates in the replication of measles virus nucleocapsids, **268,** 345
Thuriaux P. Is recombination confined to structural genes on the eukaryotic genome? **268,** 460
Tilson R. L. *see under* Tenaza R. R., **268,** 233
Timofeev P. P. *see under* Montadert L., **268,** 305
Tiong S. Y., Girton J. R., Hayes P. H. and Russell M. A. Effect of regeneration on compartment specificity of the bithorax mutant of *Drosophila melanogaster*, **268,** 435
Tischler A. S. *see under* Dichter M. A., **268,** 501
Titzsimons J. T. *see under* Evered M. D., **268,** 332
Tjernlund U. M. *see under* Klareskog L., **268,** 248
Tobe S. S., Chapman C. S. and Pratt G. E. Decay in juvenile hormone biosynthesis by insect corpus allatum after nerve transection, **268,** 728
Tobien H. *see under* Andrews P., **268,** 699
Todd I. *see under* Gingell D., **268,** 767
Tsong T. Y. *see under* Kinosita K. Jr, **268,** 438
Tsuda Y. *see under* Goto M., **268,** 755
Turner R. R. *see under* Lindberg S. E., **268,** 133
Twibell J. D. and Home J. M. Novel method for direct analysis of hydrocarbons in crime investigation and air pollution studies, **268,** 711
Tye N. C., Everitt B. J. and Iversen S. D. 5-Hydroxtryptamine and punishment, **268,** 741
Tyrer N. M. and Johnson K. A. Does electrophoresis of cockroach muscle proteins detect recognition molecules? **268,** 759
Ubbelohde A. R. New interest in carbon lamellar compounds, *news and views*, **268,** 16
Uchida S. *see under* Hotta S., **268,** 733
van Brakel J. and Heertjes P. M. Contact angle and film jumps, **268,** 44
van Deenen L. L. M. *see under* Zwaal R. F. A., **268,** 358
van Os C. H. *see under* Augustus J., **268,** 657
Vats H. O. *see under* Deshpande M. R., **268,** 614
Vedel F. *see under* Quêtier F., **268,** 365
Vigier J. P. *see under* Nottale L., **268,** 608
Villareale M. *see under* Hughes M. R., **268,** 347
Villee D. B. and Powers M. L. Effect of glucose and insulin on collagen secretion by human skin fibroblasts *in vitro*, **268,** 156
Vincze E. *see under* Kondorosi A., **268,** 525
Virtanen I. *see under* Kurki P., **268,** 240
von Borcke S. *see under* Marshall N. J., **268,** 58
von Ruden W. *see under* Bailey J., **268,** 301
Waldmann H. *see under* Phillips J. M., **268,** 641
Wang S-S. *see under* Chou J. Y., **268,** 543
Wasserman R. H. *see under* Hughes M. R., **268,** 347
Watanabe Yoshihiko. *see under* Sokawa Y., **268,** 236
Watanabe Yuji. *see under* Sokawa Y., **268,** 236
Wdowczyk J. and Wolfendale A. W. Cosmic rays and ancient catastrophes, **268,** 510
Weeks D. P., Collis P. S. and Gealt M. A. Control of induction of tubulin synthesis in *Chlamydomonas reinhardi*, **268,** 667
Weissman J. C. *see under* Benemann J. R., **268,** 19
Welsh R. M. *see under* Merigan T. C., **268,** 67
Welsh R. M. Jr and Zinkernagel R. M. Heterospecific cytotoxic cell activity induced during the first three days of acute lymphocytic choriomeningitis virus infection in mice, **268,** 646
Westoby M. Generalisation of self-thinning of plant populations — Reply, *matters arising*, **268,** 373
Wetlaufer D. B. *see under* Rose G. E., **268,** 769
Wheeler J. C. and Bash F. N. Supernovae and molecular clouds, **268,** 706
White C. M., Shultz J. L. and Sharkey A. G. Biological markers in coal and coal liquefaction products, **268,** 620
White J. Generalisation of self-thinning of plant populations, *matters arising*, **268,** 373
Whittaker R. G. *see under* Riggs M. G., **268,** 462
Whitteridge D. *see under* Ramachandran V. S., **268,** 333
Wielgus J. J. *see under* Bollenbacher W. E., **268,** 660
Wigston D. *see under* Harris A. J., **268,** 265
Wild A. Nitrate in drinking water: health hazard unlikely, *news and views*. **268,** 197
Wild A. E. and Dawson P. Evidence for Fc receptors on rabbit yolk sac endoderm, **268,** 443
Wilkie T. The Twin Paradox revisited, *news and views*, **268,** 295
Williams J. A. *see under* Chandler D. E., **268,** 659
Wimmer E. *see under* Nomoto A., **268,** 208
Winegrad S. *see under* McClellan G. B., **268,** 261
Wing E. J., Gardner I. D., Ryning F. W. and Remington J. S. Dissociation of effector functions in populations of activated macrophages, **268,** 642
Winlow W. and Benjamin P. R. Postsynaptic effects of a multiaction giant interneurone on identified snail neurones, **268,** 263
Wira C. R. and Sandoe C. P. Sex steroid hormone regulation of IgA and IgG in rat uterine secretions, **268,** 534
Witzel A. *see under* Cohen M. H., **268,** 405
Wolfendale A. W. *see under* Wdowczyk J., **268,** 510
Wolff K. *see under* Stingl G., **268,** 245
Wolff-Schreiner E. C. *see under* Stingl G., **268,** 245
Worman C. P. *see under* Burns G. F., **268,** 243
Wright H. M. *see under* Hopwood D. A., **268,** 171
Wykes T. and Johnson-Laird P. N. How do children learn the meaning of verbs? **268,** 326
Wyllie A. H., Gurdon J. B. and Price J. Nuclear localistion of an oocyte component required for the stability of injected DNA, **268,** 150
Yamamoto R. *see under* Sumino K., **268,** 73
Yang H.-Y. T. *see under* Fratta W., **268,** 452
Yasin R. *see under* Thompson E. J., **268,** 241
Yatani A. *see under* Goto M., **268,** 755
Yeats P. A. *see under* Bewers J. M., **268,** 595
Yen S. P. S. *see under* Molday R. S., **268,** 437
Yevo Y., Frangione B. and Franklin E. C. Amino acid sequence similarities between amyloid P component Clt and CRP, **268,** 56
Zambon M. *see under* Barbieri C., **268,** 318
Zeidan R. *see under* Leeder M. R., **268,** 42
Zellner B. H. *see under* Hubbard W. B., **268,** 33
Zettwoog P. *see under* Haulet R., **268,** 715
Zigler J. S. *see under* Masters P. M., **268,** 71
Zinkernagel R. M. *see under* Welsh R. M. Jr, **268,** 646
Ziskind L. *see under* Harris A. J., **268,** 265
Zohary D. *see under* Brown A., **268,** 430
Zuckerman A. J. Bed bugs and hepatitis, *news and views*, **268,** 688
Zuckerman B. Interstellar molecules, *review article*, **268,** 491
Zwaal R. F. A., Comfurius P. and van Deenen L. L. M. Membrane asymmetry and blood coagulation, **268,** 358

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